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Recent Research on Conifer Needle Diseases

Conference Proceedings
Gulfport, Mississippi
October 14 – 18, 1984

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RECENT RESEARCH ON CONIFER NEEDLE DISEASES
Conference Proceedings

October 14-18, 1984
Gulfport, Mississippi

Glenn W. Peterson
Technical Coordinator

SPONSORED BY:

The International Union of Forestry Research Organizations
Working Party on Needle Diseases

Rocky Mountain Forest and Range Experiment Station
USDA Forest Service

Southern Forest Experiment Station
USDA Forest Service

U.S. Department of Agriculture
Forest Service
Washington, D.C.

January 1986

Foreword

The International Union of Forestry Research Organizations Working Party on Needle Diseases held a conference on Recent Research on Conifer Needle Diseases in Gulfport, Mississippi, October 14-18, 1984. The conference consisted of presentation of papers and discussions and observation of experiments in field plantings and nurseries. The papers covered research on infection, epidemiology, ecology, resistance, taxonomy, and control. There were 16 participants, 6 from outside North America. The participants noted an increase in research on resistance to needle pathogens since the last meeting of the Working Party in Sarajevo, Yugoslavia, in 1980.

During the business meeting, the objectives of IUFRO Working Parties were reviewed and the conclusion reached that the activities of this Working Party were consistent with the objectives and that the working party should be continued.

After the conference a 4-day excursion was conducted to acquaint participants with a range of research activities in forestry in the southern United States. Visits were made to the Natchez Research Center of the International Paper Company and to USDA Forest Service Laboratories at Stoneville, Mississippi; Alexandria, Louisiana; and Gulfport, Mississippi. Types of research being conducted were discussed by laboratory personnel, then visits were made to field research sites.

Rapid publication of these proceedings was due largely to the excellent efforts of the authors (and the typists!) in preparing the manuscripts, many of which we received camera-ready. Since papers are, essentially, being printed as received, each contributor is responsible for the accuracy of his or her paper; opinions expressed by the authors may not necessarily reflect the policy of the U.S. Department of Agriculture.

We owe many thanks to Albert Kais for making arrangements for meeting facilities, housing, and field trips. We appreciate the time spent by personnel at the various forestry centers in explaining and showing us their work. We thank Rose Cefkin for compiling the conference papers for publication.

GLENN W. PETERSON, Chairman
IUFRO Working Party on Needle Diseases

Peterson, Glenn W., tech. coord. Recent research on conifer needle diseases: Proceedings of the International Union of Forestry Research Organizations Working Party on Needle Diseases Conference; 1984 October 14-18; Gulfport, MS. General Technical Report WO-50. Washington, DC: U.S. Department of Agriculture, Forest Service; 1985: 106 p.

Results of current research on foliage diseases of conifers in several countries were presented in 15 papers. The results included aspects of infection, genetic resistance, ecology, epidemiology, taxonomy, and control.

Keywords: Tree diseases, conifers, foliage diseases, needle cast

Cover: *Bifusella linearis*--
on needles (see p. 80).

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Contents

Histological Studies of *Scirrhia acicola* (Dearn.) Siggers and Other Needle-Inhabiting Fungi on Longleaf and Loblolly Pines[1]

F. F. Jewell, Sr.[2]

Abstract.--Scirrhia acicola (Dearn.) Siggers, the brown spot needle blight fungus, caused similar host reactions in field-grown needle tissue of longleaf pine, Pinus palustris Mill., and loblolly pine, P. taeda L. The major response with typically symptomatic samples was in the mesophyll, where a specific, sharply defined cellular collapse occurred, while little or no pathological effect was evident in the endodermis or underlying tissues. In general, hyphae of S. acicola were sparse in the affected tissue areas of both pine species, but profuse and localized subepidermally under perithecial and conidial stroma. This overall response was quite indicative of a toxin in the host/parasite relationship. Some loblolly samples bearing symptoms similar to those considered typical for S. acicola had, in addition to the specific cellular collapse, anatomical abnormalities in the endodermis that extended into the transfusion tissue. Associated consistently with S. acicola on the host needles were species of Lophodermium, Pestalotia, Leptostroma, and Fumago.

INTRODUCTION

Although the brown spot needle disease (Scirrhia acicola (Dearn.) Siggers) is prevalent throughout the South on several species of Pinus (Siggers 1944), the disease is of consequence primarily on longleaf pine (P. palustris Mill.) (Siggers 1932, 1944). Loblolly pine (P. taeda L.) is a common host of S. acicola (Hedgcock 1929), and a prevalent dieback of needles of this host has been reported (Boyce 1952). There are numerous publications concerning S. acicola: Dearness (1926, 1928), Hedgcock (1929), Verrall (1934), Siggers (1939, 1944), Wolf and Barbour (1941), Henry (1954), Snow (1961), and Setliff and Patton (1974). These are just a portion of a large literature dealing with the brown spot disease, its taxonomy, and its epidemiology. It was not until recently that attention was given to studies on the anatomical effects of S. acicola on host pine needles, and on particular fungal associates of S. acicola (Jewell 1981, 1983, 1984).

The present paper is a summation of present and past (Jewell 1983) observations on the pathological anatomy of S. acicola infected longleaf and loblolly pine needles, as well as of fungi consistently associated with S. acicola on the pine hosts studied.

EXPERIMENTAL METHODS

Fascicled needles were collected from field-grown loblolly and longleaf pines in south Mississippi. Samples were taken from green (living) and dead needle areas. The only basis for sampling dead needles was color. Brown needles were considered dead, and no time sequence from green to brown was used. The green tissue contained typical bar-spots (Siggers 1944). In addition, samples of loblolly needles bearing yellow or yellow-brown spots were collected. The dead tissue usually exhibited no discernable symptoms other than water-soaked appearing areas that may have been typically symptomatic when green. Approximately, 30 individuals of each pine species were sampled. Histological processing of tissue for paraffin sectioning and observation was as previously reported (Jewell et al. 1962). Fresh mounts supplemented study of spore characteristics.

[1]Paper presented at the IUFRO Working Party Conference on Recent Research on Conifer Needle Diseases [Gulfport, Miss., U.S.A., October 14-18, 1984].

[2]F. F. Jewell, Sr., is Professor of Forestry at Louisiana Tech. University, Ruston, LA 71270 U.S.A.

RESULTS

Green Tissue

Observations of typical S. acicola bar-spot symptom areas of loblolly and longleaf needles indicated a similar pathological anatomy developed in these hosts effected by the pathogen (fig. 1, A-D). The major tissue abnormality was in the mesophyll, where a drastic collapse of cells was evident (fig. 1, B, D). This cellular collapse caused the individual mesophyll cell to become flattened and produce, in longitudinal view, a lattice- or ladder-like appearance with large intercellular spaces in the affected area. Usually, hypodermal, endodermal, transfusion, or vascular tissue exhibited little or no abnormality in the symptomatic areas. Frequently, normal-appearing endodermal or hypodermal cells were in direct contact with collapsed mesophyll. There was sharp definition, also, of unaffected and affected mesophyll at the edges of the

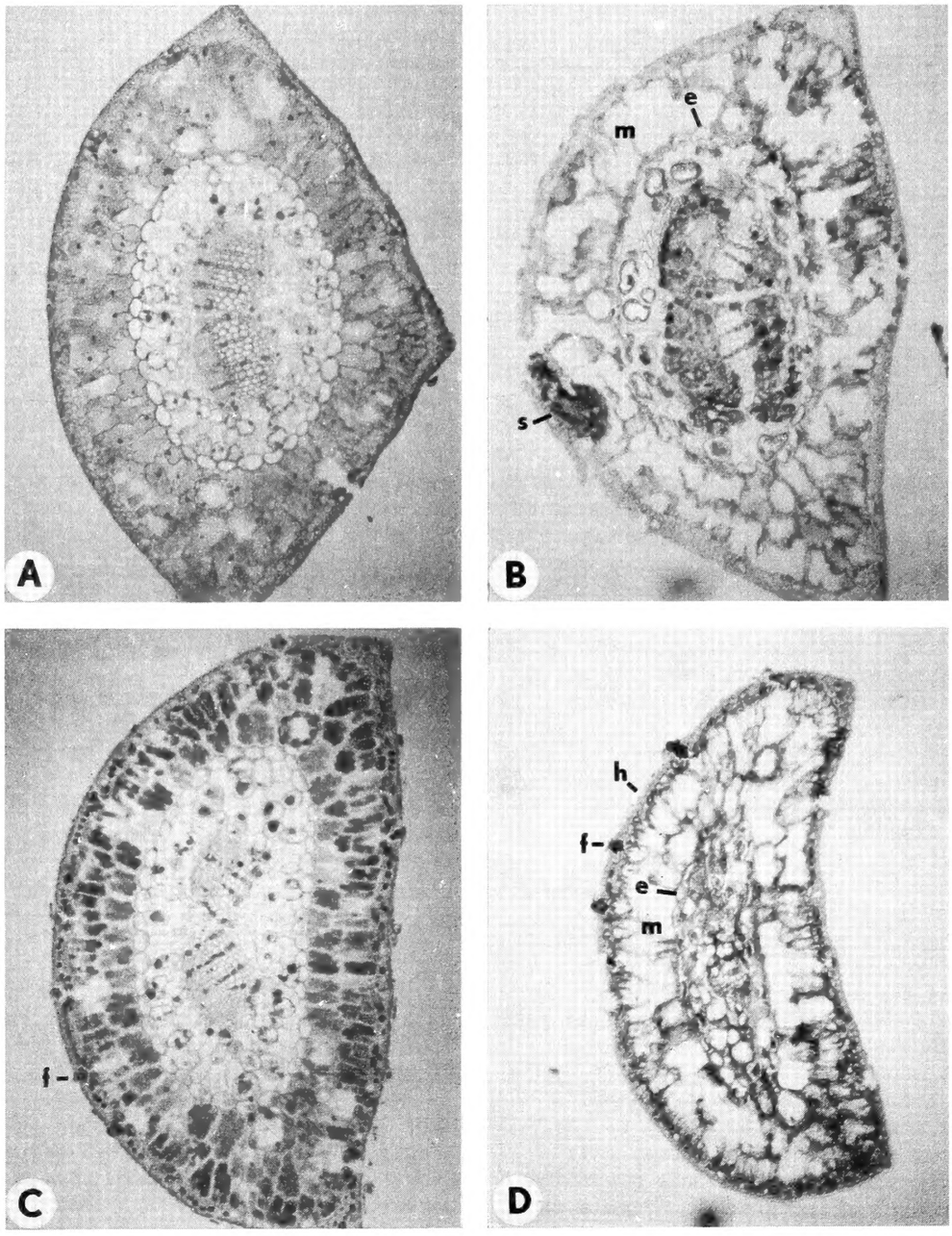

Figure 1.--Transverse sections of needles of longleaf and loblolly pines. A. A normal fascicled needle of longleaf pine. 75x. B. Section through a bar-spot symptom of S. acicola on a longleaf pine needle. Note collapsed mesophyll (m) and delimiting endodermis (e) and ascervulus (s). 75x. C. A normal fascicled needle of loblolly pine. A Fumago sp. (f) is plugging some stomata. 75x. D. Section through a bar-spot symptom of S. acicola on a loblolly pine needle. Note collapsed mesophyll (m) and delimiting endodermis (e) and a Fumago sp. (f) plugging stomata and superficial hypha (h). 75x.

symptomatic tissue. Here, collapsed cells were in direct contact with normal mesophyll cells.

The presence of S. acicola in affected tissue was sparse. This substantiates the report by Verrall (1934) of limited hyphae in the mesophyll of bar-spots. In the present work hyphae of S. acicola were very difficult to observe, but occasionally were present in the intercellular spaces between mesophyll cells or intracellular in collapsed mesophyll cells. The scant presence of S. acicola hyphae was far out of proportion to the amount of affected host tissue. Rarely were hyphae observed in other than the areas of cell collapse. Similar findings were reported for P. radiata/Dothistroma pine relationship by Gadgil (1967).

Hyphae were abundant in affected tissue areas that were to support sporulation. Large, dark brown, short-celled hyphae formed in the outer mesophyll and between the hypodermis and epidermis. Stromata were formed in such areas; these stromata produced ascervuli and conidia as described by Siggers (1944). No ascostromata were observed on the green symptomatic tissue studied.

Loblolly pine needles bearing yellow or yellow-brown spot symptoms were an exception to the general host effect caused by S. acicola. These symptomatic areas had, in addition to the sharply delimited collapsed mesophyll, lesion-like abnormalities extending through the endodermis into the transfusion tissue. In longitudinal view the affected area was saucer-shaped and often had periderm-like cells delimiting the inner extent into the transfusion tissue. Intracellular and intercellular hyphae, identity unknown, were common and often profuse. Frequently, tissue abnormalities of this type subtended stroma of a Lophodermium spp. A direct pathogen effect is uncertain at present. However, S. acicola sporulation was not observed on this type of symptomatic tissue.

Dead Tissue

Considerable deterioration of all tissues occurred in dead needle samples. The mesophyll collapse was evident, but the endodermis, transfusion, and vascular tissues exhibited various stages of degeneration. In some cases, the vascular tissues appeared viable while all other tissue systems were nonfunctional. In other samples, all tissue systems appeared dead or nonfunctional. Unfortunately, it was not possible to sequence tissue deterioration in the dead samples, as collections of these were random and nonspecific as to stage or length of time the needles had been brown or dead.

In contrast to green tissue, intracellular and intercellular hyphae were prevalent throughout all tissue systems of dead samples. The identity of the hyphae was uncertain except in areas associated with sporulation. Both the asexual and sexual stages of S. acicola were observed, with the latter being exclusively associated with dead tissue. The relation of these structures and longleaf pine has been adequately described (Siggers 1944, Jewell 1983). No differences were observed for loblolly pine.

Associated Fungi

Several genera of fungi were consistently found in conjunction with S. acicola in the samples observed. The genera represented were Fumago, Pestalotia, Leptostroma, Lophodermium, and Hypoderma. Of these species of Fumago, Pestalotia, and Lophodermium were associated with S. acicola on green and dead samples of both longleaf and loblolly. On dead tissue, a Lophodermium sp. was probably the common associate. A Leptostroma sp. was more prevalent on loblolly. A Fumago sp. was found occasionally on green but not dead longleaf samples. On loblolly, a Fumago sp. was consistently observed plugging stomata and superficially on green and dead symptomatic tissue and to a lesser extent on longleaf. Of particular interest was the presence of a Fumago sp. on all samples of nonsymptomatic (normal) green loblolly observed (fig. 1, C). This was not true of the other fungal associates. A Pestalotia sp. was most prevalent on green samples, often sporulating in the bar-spot area. A Hypoderma sp. was an infrequent inhabitant of loblolly and longleaf and, at present, considered of little significance.

DISCUSSION

The reaction of loblolly and longleaf pine needle tissue to infection by S. acicola appears as a severe but limited collapse of the mesophyll cells. This is true even with a very limited presence of S. acicola hyphae. This rather intensive host response, occurring in the absence of or with meager presence of the hyphae of S. acicola, strongly suggests the possibility of a toxin being produced in the host/pathogen interaction. This theory is supported in part by research of Gadgil (1967). A somewhat similar host reaction was described by Peterson and Walla (1978) for Austrian and ponderosa pines infected by Dothistroma pini, although extensive hyphal development of the pathogen was observed. The role of a toxin was not discussed. However, the hypothesis of an S. acicola - toxin-host relationship is strengthened by the establishment by Shain and Franich (1981) of Dothistroma blight symptom induction by the substance dothistromin. As Scirrhia acicola and Dothistroma (Scirrhia) pini are very close taxonomically (differ in color of conidia) and have similar effects on their respective hosts, it is reasonable to assume an S. acicola toxin exists in a manner similar to dothistromin. This hypothesis needs investigation.

The role of the fungi associated with S. acicola in the development of the brown spot disease is, at present, uncertain. The more common associates were observed along with S. acicola on green and dead tissue. At present, no pathological host damage can be attributed to any of the observed fungi other than S. acicola. There is suggestion that Lophodermium sp. causes certain symptoms on loblolly needles, but this is not

presently valid. The constant presence of Fumago sp. on green non-symptomatic and symptomatic loblolly is interesting. The significance of this occurrence, particularly the stomatal plugging, is not understood. Similar uncertainty was expressed by Parris and Killebrew (1969). These same authors stated the situation with fungal associates of S. acicola quite well: "The role of extraneous fungi, especially Fumago sp., in the brown spot complex on loblolly pine remains a fascinating and unsolved problem." We could include in their assessment the genus Lophodermium and longleaf pine.

Our research indicates that there is more than one fungus involved in causing brown spot. S. acicola is certainly a major contributor to the disease, but there is a distinct probability that a disease complex exists of S. acicola plus one or two other species of fungi being responsible for what is known as brown spot needle disease on loblolly and longleaf pine.

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Disease Progress of *Scirrhia acicola* in Single and Mixed Family Plantings of Resistant and Susceptible Longleaf Pine[1]

Margene M. Griggs and Robert A. Schmidt[2,3,4]

Abstract.--Open-pollinated families of longleaf pine (Pinus palustris) resistant and susceptible to brown spot needle blight (BSNB) were planted in single and mixed family plots in two southern states. Epidemics were initiated by placing infected longleaf pine needles on susceptible longleaf plants in the center of the plots. Lesion numbers and percent needle dieback were measured at 6-week intervals for two growing seasons and disease progress curves constructed. Resistant families delayed the onset of dieback 5 to 7 weeks and had lower levels of maximum needle dieback. The rate of percent needle dieback increase varied by distance from the initial infection focus (range = 0.09 - 0.23 m/week); however, there were no significant family or location differences. The mixed family plots did not limit BSNB development in time or space when compared to the susceptible family plots, as factors favoring within-plant transmission predominated over those favoring between-plant transmission.

INTRODUCTION

Brown spot needle blight (BNSB), caused by Scirrhia acicola (Dearn.) Siggers, attacks longleaf pine (Pinus palustris Mill.) in its juvenile grass stage. The disease reduces plant vigor through defoliation, delays the onset of rapid height growth, and predisposes seedlings to early death. Such problems lead to extended rotation periods, irregular or inadequate stocking, and nonuniform stand conditions in plantations.

Longleaf pine possesses heritable resistance to BSNB (Derr 1963, Snyder and Derr 1972); but little is known about the nature of this resistance. Thus, host-pathogen responses in both resistant and susceptible trees should be characterized. The comparison of disease progress curves for single family plantings of resistant and susceptible longleaf pines can provide useful information.

Variety mixtures and multilines have lowered disease levels and increased yields in small grains such as barley (Wolfe and Barrett 1977, Wolfe 1978), wheat (Groenewegen and Zadoks 1979), and oats (Browning and Frey 1969, Frey et al. 1973). In addition, several workers (Berger 1973, Browning et al. 1962, Jensen and Kent 1963, Leonard 1969, Rothman and Frey 1953) found that susceptible plants in mixed plantings with resistant plants were damaged less than were susceptible plants in pure (or single) stands. In longleaf pine management there is the opportunity to mix resistant and susceptible open-pollinated families (these can be considered "varieties"). The comparison of disease progress curves from such mixed plantings could provide insight into possible deployment strategies for resistant and susceptible longleaf pine families. The objectives of this study were to (1) identify and compare quantitative parameters of disease progress curves for artificially induced BSNB epidemics in single family field plantings of resistant or susceptible longleaf pines; (2) determine the effect of 50:50 mixtures of resistant or susceptible families on these disease progress parameters; and (3) compare the single and mixed family disease progress parameters at two geographic locations.

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[1]Paper presented at the IUFRO Working Party Conference on Recent Research on Conifer Needle Diseases [Gulfport, Miss., U.S.A., October 14-18, 1984].

[2]Margene M. Griggs is Plant Geneticist, USDA Forest Service, Southern Forest Experiment Station, Gulfport, MS 39503 U.S.A., and Robert A. Schmidt is Professor, School of Forest Resources and Conservation, University of Florida, Gainesville FL 32601 U.S.A.

[3]Portion of a dissertation submitted by the senior author in partial fulfillment of the requirements for the Ph.D. degree, University of Florida.

[4]The cooperation of Jim McConnell, USDA Forest Service, Region 8, and International Paper Company, Bainbridge, Ga., is gratefully acknowledged.

MATERIALS AND METHODS

Two resistant and two susceptible open-pollinated longleaf pine families (Derr 1971, Snyder 1977, Snyder and Hamaker 1978) were planted in single and mixed family plots; each plot consisted of 64 trees (fig. 1A). One study location was in south Mississippi and the other in southwest Georgia. The mixed family plots were a 50:50 mix of alternately planted individuals from a resistant and a susceptible family (fig. 1B). Four family mixture combinations resulted. Three of the families were from USDA Forest Service (Region 8) plus-tree selections in south Mississippi, while the fourth family was from a plus-tree selection in South Carolina. The control was a susceptible bulk seedlot. Each location was a randomized complete block design with four replications.

Epidemics were initiated in all but the susceptible control plots in May 1980, by placing severely infected longleaf pine needles on susceptible longleaf plants (disease spreaders) in the center of the plots. Disease was measured as (1) number of lesions on a random sample of 10 fascicles/tree, and (2) percent needle dieback on a sample of 20 fascicles/tree. Measurements were at 6-week intervals during two growing seasons at four distances from the center of plot: 0.3 m, 1.2 m, 2.1 m, and 3.0 m. Disease progress curves were developed from these measurements. Precipitation was monitored at each planting location by a recording rain gauge.

For the comparison of epidemics, Kranz (1974) described several elements characteristic of disease progress curves. Curve elements are thought to represent certain biological events integrated in the disease progress curves and are regarded as gauges for the effects of time. Even more important, they are the basis for quantitative comparisons of epidemics. In this study, the following epidemiological progress curve elements were determined for each tree:

YMAX - The maximum amount of needle dieback observed during the two growing seasons.

TBEG - The time (wk) when needle dieback was first observed.

TMAX - The time (wk) when YMAX was observed.

YRATE - The average apparent rate of percent needle dieback increase per week. Calculated as described by Vanderplank (1963) and Starkey (1977).

Averaged plot means were calculated for each distance for the two resistant families ("resistant"), for the two susceptible families ("susceptible"), and for the four family mixtures ("mixed family" or "family mixtures"). Averaged plot means were statistically analyzed (ANOV) for all disease variables with tests of significance at the 0.05 level.

RESULTS

Since needle dieback is caused by the coalescing of lesions, lesion numbers were statistically analyzed at 18 weeks after epidemic initiation to minimize bias from dieback (needle dieback was first observed at 24 weeks). There were no significant differences among families for lesion numbers 18 weeks after epidemic initiation. There was a significant location effect: seedlings in Georgia had three times more lesions than seedlings in Mississippi.

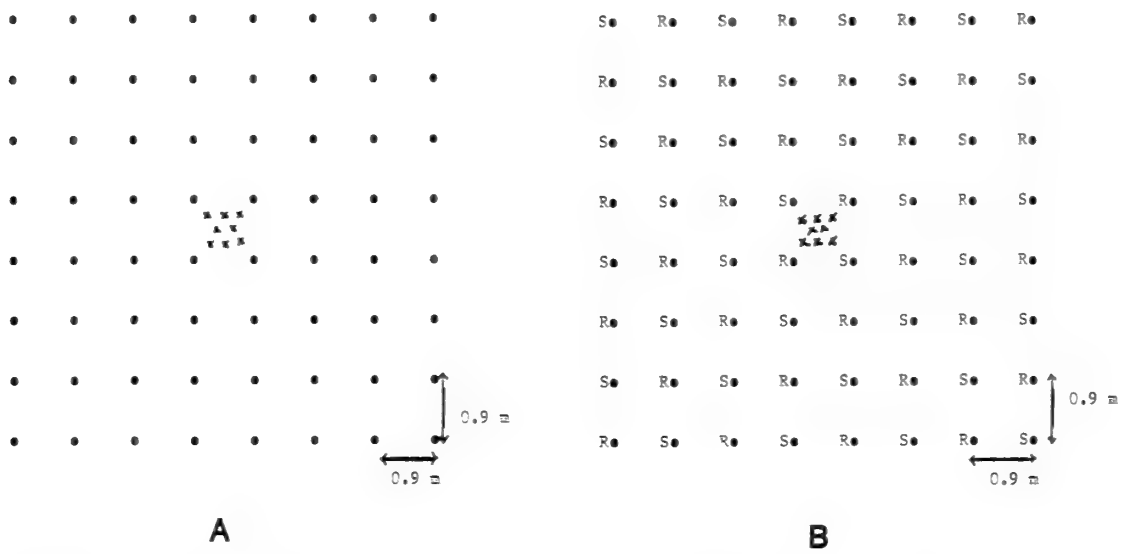

Figure 1.--Single family (A) and mixed family (B) plots, each consisting of 64 longleaf pine seedlings. Epidemics of Scirrhia acicola were initiated in center of plots on spreader trees (x). R = resistant family tree; S = susceptible family tree.

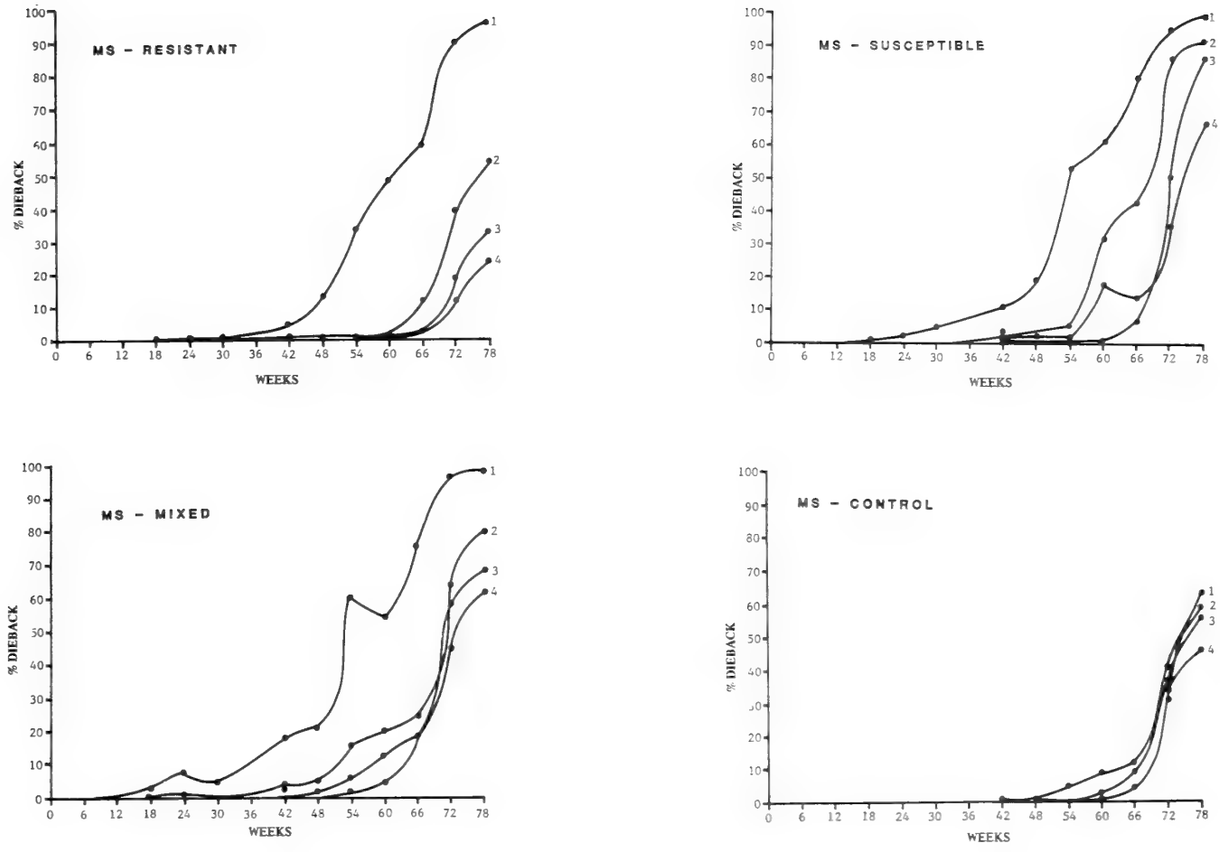

Figure 2.--Disease progress curves of percent needle dieback at four distances from the initial infection focus in resistant, susceptible, mixed family, and control plantings of longleaf pine exposed to Scirrhia acicola for two growing seasons in Mississippi. 1 = 0.3 m, 2 = 1.2 m, 3 = 2.1 m, 4 = 3.0 m.

At the end of two growing seasons the progress curves of percent needle dieback showed that disease levels were much greater in Georgia than in Mississippi (figs. 2 and 3). Percent needle dieback was <10% during the first growing season (week 42) in Mississippi. Needle dieback was not observed on the control seedlings until week 42 (fig. 2). In Georgia, however, needle dieback exceeded 10% by week 24 (fig. 3), and the control plots showed needle dieback beginning at week 18.

Disease progress fluctuated, particularly during the second growing season in Georgia (fig. 3). The fluctuations were caused by excessive loss of dead (BSNB-killed) needle fascicles followed by the rapid development of new needle growth or "flushes." The latter initially showed no BSNB dieback; hence percent dieback decreased by the next measurement.

Maximum Amount of Needle Dieback (YMAX)

At both locations, YMAX decreased as distance increased from the initial infection focus. Averaged over distances, YMAX was significantly less for the resistant families than for the susceptible families (fig. 4). In Mississippi, family effects were significant at all distances from the center of plots except at the closest distance, 0.3 m. Inoculum levels were probably so high at 0.3 m that the resistance or tolerance of all families was overcome. In Georgia, significant family differences were observed only at the farthest distance, 3.0 m. Family mixtures did not result in significantly reducing YMAX when compared to the susceptible families at either location (fig. 4).

Time of Beginning Needle Dieback (TBEG)

At both locations, the values for TBEG increased as distance increased from the initial infection focus (fig. 5). In Mississippi, family effects were significant at the farthest distance, while in Georgia differences were observed in the outer three distances from the initial infection focus. Resistant families, on the average, delayed the onset of needle dieback by 5 weeks in Mississippi and by 7 weeks in Georgia when compared to susceptible families. Family mixtures did not significantly differ from susceptible families in either Mississippi or Georgia (fig. 5).

Time of Maximum Needle Dieback (TMAX)

There were no significant family or location effects for TMAX.

Rate of Percent Needle Dieback Increase (YRATE)

In Mississippi and Georgia, there were no significant differences for YRATE (range = 0.09-0.23 m/week). Though not significant, the

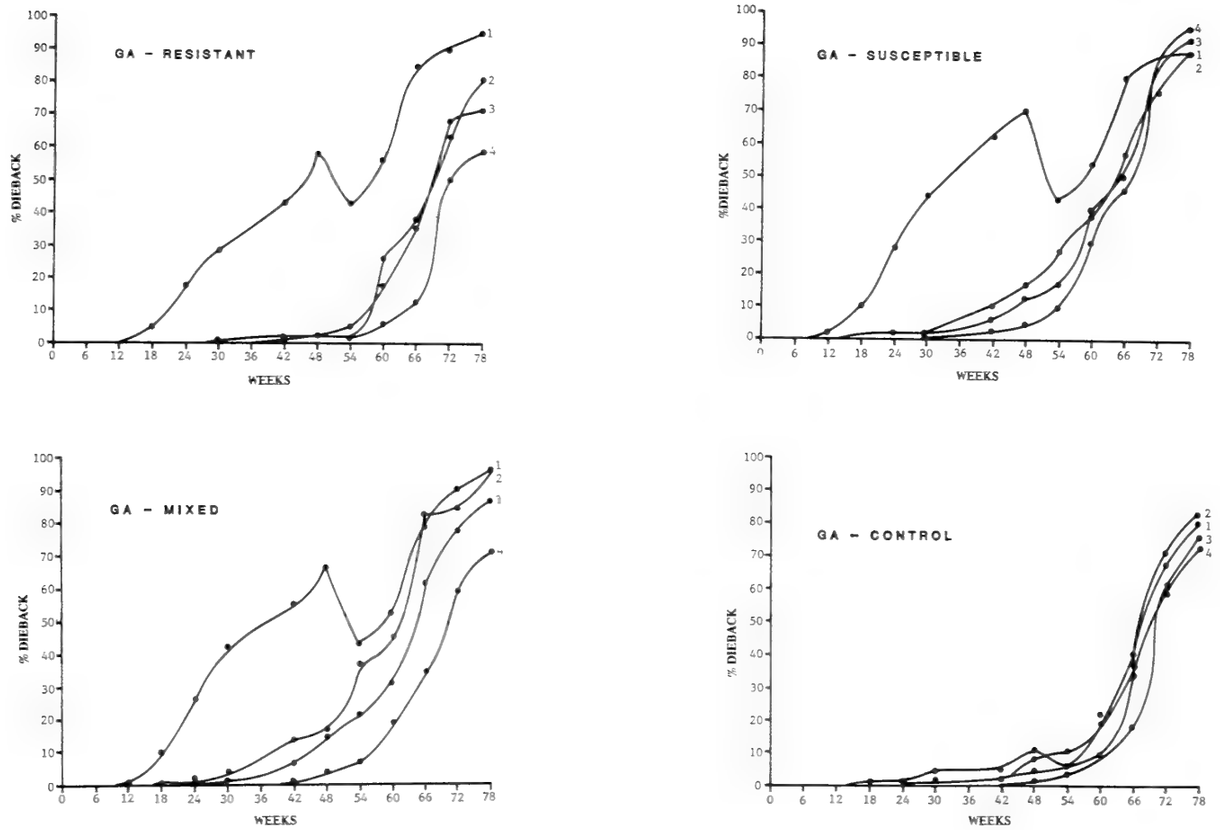

Figure 3.--Disease progress curves of percent needle dieback at four distances from the initial infection focus in resistant, susceptible, mixed family, and control plantings of longleaf pine exposed to Scirrhia acicola for two growing seasons in Georgia. 1 = 0.3 m, 2 = 1.2 m, 3 = 2.1 m, 4 = 3.0 m.

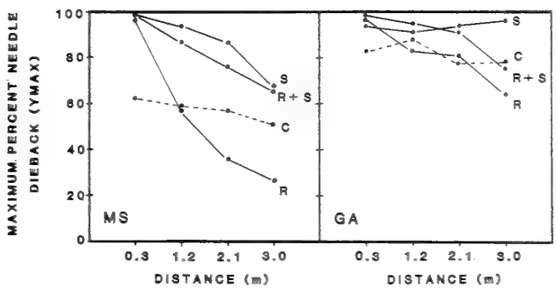

Figure 4.--Maximum percent brown spot needle dieback (YMAX) by distance from initial infection focus for resistant (R), susceptible (S), mixed family (R+S), and control (C) plantings of longleaf pine after two growing seasons in Mississippi (MS) and Georgia (GA).

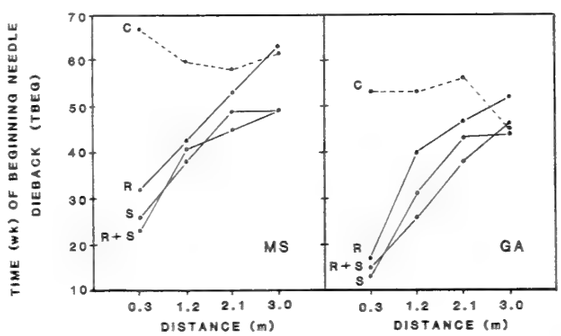

Figure 5.--Time (wk) of beginning brown spot needle dieback (TBEG) by distance (m) from initial infection focus for resistant (R), susceptible (S), mixed family (R+S), and control (C), plantings of longleaf pine in Mississippi (MS) and Georgia (GA).

resistant families consistently had smaller YRATE values. Family mixtures did not reduce the YRATE when compared to the susceptible families. In fact, the family mixtures had greater YRATE values. Over all families, YRATE decreased as distance from initial infection focus increased.

DISCUSSION

Even though the quantity and quality of initial inoculum was similar, BSNB severity levels were much higher in Georgia than in Mississippi.

This was primarily due to a drought that occurred in south Mississippi from May-October 1980. The lack of rain prevented dispersal of the rain-splash disseminated conidia of S. acicola and subsequent infection during the first growing season.

Resistance to BSNB was expressed primarily as a delay in the onset of needle dieback (TBEG). Both resistant and susceptible longleaf families had similar YRATE values, but the resistant families had larger TBEG values and smaller YMAX values. The "resistance" appears to be a tolerance phenomenon. Resistant and susceptible families had similar numbers of lesions, but the susceptible families expressed needle dieback much earlier--5 to 7 weeks. Once begun, needle dieback proceeded at a fairly constant rate in all families. Perhaps each family has a tolerance threshold to a toxin produced by S. acicola. Once this threshold is reached, resistance is overcome and needle tissue begins to die. In histopathological examinations of S. acicola in severely damaged longleaf pine needles, Jewell (1983) observed limited development of S. acicola hyphae in symptom areas. Jewell suggested that a toxin may be produced by S. acicola. Shain and Franich (1981) found a toxin (dothistromin) produced by the closely related S. pini (Dothistroma needle blight fungus). The toxin caused typical necrotic red band symptoms on P. radiata.

Using family mixtures (variety mixtures) did not succeed in reducing disease levels in longleaf pine. This is attributed, in part, to the physical form of the longleaf pine grass stage seedling. Each seedling supports a very large number of three-needle fascicles, which provides an enormous surface area with the same genotype for S. acicola to infect and colonize. Thus, inoculum levels can increase rapidly on an individual seedling (auto-infection) and a small epidemic can develop independently, unaffected by the presence or absence of a resistant neighbor tree (Heybroek 1982). By comparison, in small grains, an individual plant is small and narrow. Also, the individual cultivar components of small grain mixtures or multilines are inbred, such that specific cultivars are susceptible to certain strain(s) of the pathogen. Spores produced on one individual are incapable of infecting a neighboring plant of a different cultivar. Thus, the spore cloud is effectively diluted.

Another factor contributing to the poor performance of family mixtures in longleaf pine is the mode of dispersal of S. acicola conidia. Conidia of S. acicola are rain-splash disseminated. This further encourages auto-infection in that it limits dispersal to the immediate vicinity of the plant and limits allo-infection (between-plant transmission) to those trees nearby. Thus, within the mixed family plots in this study, the susceptible trees each ultimately acted as a disease spreader, overcoming the resistance of the resistant neighbor tree.

A third factor perhaps responsible for the failure of family mixtures to reduce disease levels was the tree-to-tree variability associated with the open-pollinated nature of the longleaf pine seedlings: a resistant parent always has a certain proportion of susceptible progeny.

CONCLUSIONS

Resistance to BSNB in open-pollinated longleaf pine grown in Mississippi and Georgia was expressed primarily as a delay in the onset of needle dieback. Some families were very tolerant of lesions while others were not and expressed needle dieback quite early in the epidemic.

Comparative epidemiological parameters of the percent needle dieback progress curves were helpful in comparing families. The parameter TBEG (time of beginning needle dieback) was 5-7 weeks later for resistant families compared to susceptible families. Resistant families always had lower YMAX (maximum percent needle dieback).

Rates of needle dieback increase per week (YRATE) did not differ.

Very little family x location interaction occurred. Of six variables measured, only YMAX and TMAX showed any significance for this interaction.

Mixing resistant and susceptible open-pollinated families in longleaf pine does not appear to be a useful strategy to slow the BSNB epidemic in time or space, at least not for the families used in this study.

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Brown Spot Needle Blight Susceptibility of Longleaf Pine Seed Sources in Georgia and Florida[1]

John F. Kraus[2]

Abstract.--Brown spot needle blight (Scirrhia acicola) was scored on 5-year-old longleaf pine (Pinus palustris) trees from 20 sources in Georgia, Florida, and Alabama and planted at five locations in Georgia and two in Florida. The results showed that the most southerly seed source, from Glades County, Florida, was highly susceptible. The variation in infection among the other 19 seed sources was random and not significantly different among sources.

Selection for resistance to the brown spot needle blight fungus should be based on individual tree selection and progeny testing.

INTRODUCTION

Longleaf pine (Pinus palustris Mill.) was a major component of the virgin southern pine forest. It remains so, despite 50 years of benign neglect because of lack of knowledge of how to consistently obtain adequate natural and artificial regeneration. It also had to compete for the favor of foresters with its faster growing and easily regenerated associates, slash pine (P. elliottii Engelm.) and loblolly pine (P. taeda L.). Experience and research have solved most of the artificial (Mann 1969) and natural (Croker and Boyer 1975) regeneration problems; but losses in annual wood production, due to mortality and delayed growth associated with infection by the brown spot needle blight fungus, (Scirrhia acicola (Dearn.) Siggers) were estimated to be 453,000 m^3 (16 million cubic feet) (Phelps et al. 1978).

The brown spot needle blight fungus can be controlled in nurseries, small plantings, and on individual trees by foliar application of Bordeaux mixture, chlorothalonil, or benomyl (Kais 1975, Kais et al. 1981). Growth losses due to brown spot could be reduced if seed sources that have genetic resistance to this fungus and good growth characteristics could be found, as they have been for fusiform rust (Cronartium quercuum (Berk.) Miyabe ex Shirai f. sp. fusiforme) of loblolly pine (Wells and Wakeley 1966). This paper compares the brown spot needle blight found in a study of seed sources of longleaf pine planted in Georgia and Florida.

MATERIALS AND METHODS

Cones were collected in 1967 and 1968 from a minimum of 20 trees at 14 locations in Georgia, 5 in Florida, and 1 in Alabama (fig. 1). At least 20 cones were collected from each tree. Seedlings produced from these source collections were planted at five locations in Georgia and two in Florida in 1970 (fig. 1). Six replications of 25-tree square plots were planted at each location.

Percentage of brown spot needle blight was scored in 1975 on all surviving trees with a scoring system of 1 = 0-19% through 5 = 80-100% needle infection. The resulting data were evaluated by an analysis of variance of plot mean-infection scores. The seed sources were also grouped according to their physiographic origin and analyzed to see if sources from any particular physiographic province were more susceptible (or less so) to infection than sources from other provinces.

Correlations were calculated between infection and 5th-year average annual height growth.

RESULTS

There were significant differences in percentage of needle infection among seed sources at six of the planting locations (table 1), despite infection levels that usually averaged

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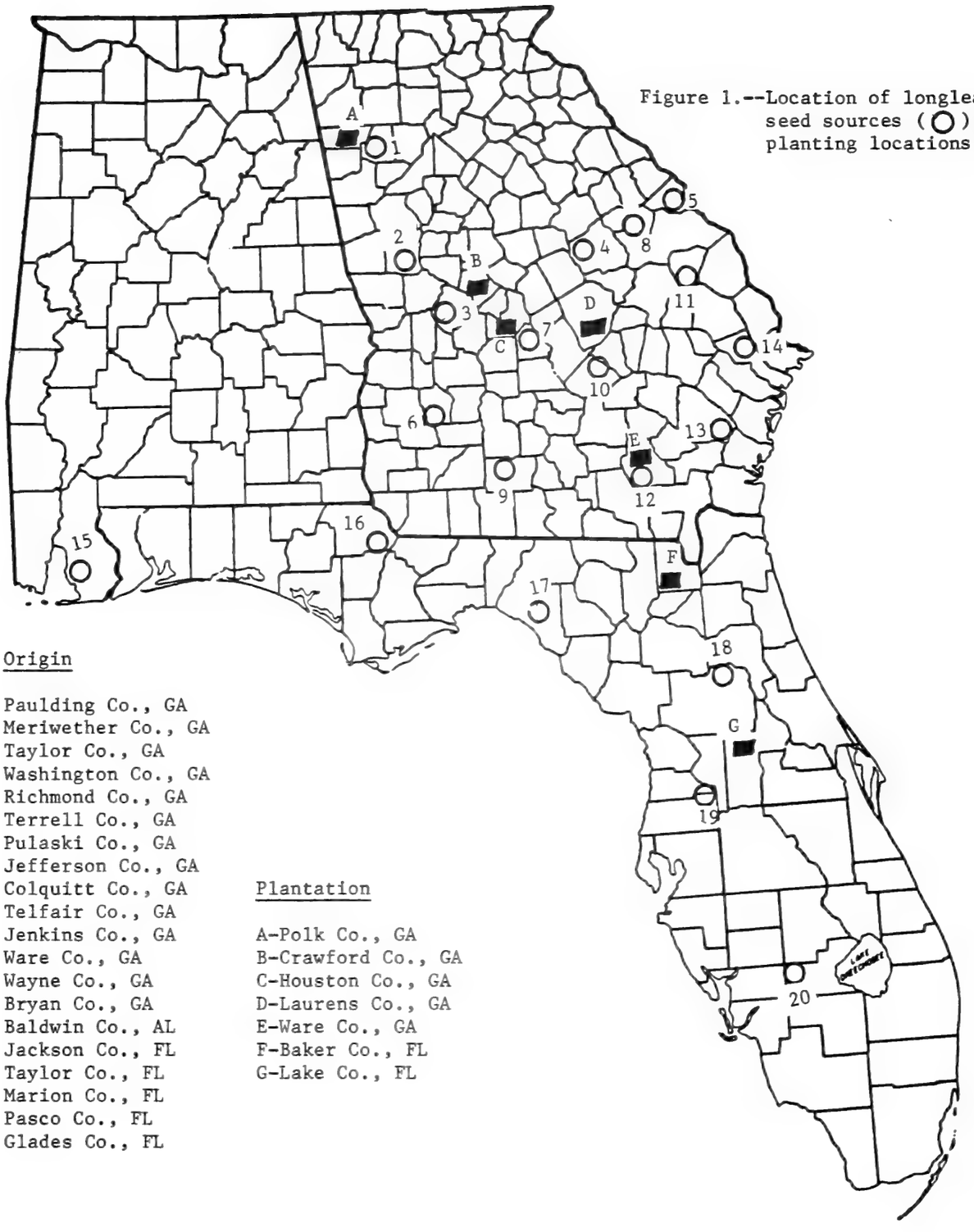

Figure 1.--Location of longleaf pine seed sources (○) and planting locations (■).

Source Number	Origin
1	Paulding Co., GA
2	Meriwether Co., GA
3	Taylor Co., GA
4	Washington Co., GA
5	Richmond Co., GA
6	Terrell Co., GA
7	Pulaski Co., GA
8	Jefferson Co., GA
9	Colquitt Co., GA
10	Telfair Co., GA
11	Jenkins Co., GA
12	Ware Co., GA
13	Wayne Co., GA
14	Bryan Co., GA
15	Baldwin Co., AL
16	Jackson Co., FL
17	Taylor Co., FL
18	Marion Co., FL
19	Pasco Co., FL
20	Glades Co., FL

Plantation
A-Polk Co., GA
B-Crawford Co., GA
C-Houston Co., GA
D-Laurens Co., GA
E-Ware Co., GA
F-Baker Co., FL
G-Lake Co., FL

Table 1.--Average of brown spot needle blight scores for longleaf pine 5 years after planting in a seed source study in Georgia and Florida.

Origin		Physiographic province	Plantation location							Means	
County	State		Polk Co., GA	Crawford Co., GA	Houston Co., GA	Laurens Co., GA	Ware Co., GA	Baker Co., FL	Lake Co., FL	Origin	Province
			Infection scores[1]								
		Piedmont									1.54
Paulding	GA		1.56	1.26	1.17	1.32	1.60	1.73	2.64	1.61	
Meriwether	GA		1.48	1.27	1.10	1.33	1.44	1.42	2.30	1.48	
		Sandhills									1.47
Taylor	GA		1.59	1.22	1.14	1.41	1.24	1.70	2.02	1.47	
Washington	GA		1.52	1.22	1.12	1.40	1.22	1.84	2.14	1.49	
Richmond	GA		1.56	1.24	1.17	1.22	1.09	1.53	2.27	1.44	
		Upper Coastal Plain									1.47
Terrell	GA		1.71	1.24	1.09	1.24	1.27	1.43	2.12	1.44	
Pulaski	GA		1.66	1.15	1.26	1.25	1.27	1.58	1.92	1.44	
Jefferson	GA		1.59	1.39	1.17	1.57	1.35	1.47	2.13	1.52	
		Lower Coastal Plain									1.59
Colquitt	GA		1.69	1.16	1.26	1.58	1.57	1.69	2.63	1.66	
Telfair	GA		1.56	1.16	1.05	1.57	1.31	2.08	2.62	1.62	
Jenkins	GA		1.56	1.21	1.04	1.36	1.46	1.90	1.84	1.48	
		Flatwoods									1.52
Ware	GA		1.54	1.20	1.20	1.26	1.30	1.56	2.58	1.52	
Wayne	GA		1.55	1.12	1.35	1.36	1.55	1.58	2.70	1.60	
Bryan	GA		1.56	1.32	1.24	1.21	1.28	1.40	1.99	1.43	
		Alabama and Florida									1.73
Baldwin	AL		1.81	1.28	1.28	1.16	1.36	1.86	1.84	1.51	
Jackson	FL		1.58	1.22	1.16	1.41	1.29	1.50	1.76	1.42	
Taylor	FL		1.88	1.30	1.15	1.56	1.56	1.44	2.22	1.59	
Marion	FL		1.84	1.32	1.10	1.60	1.60	1.60	2.92	1.71	
Pasco	FL					1.44			1.65	1.55	
Glades	FL		2.59	1.50	1.39	2.47	2.77	2.90	3.60	2.46	
Significance of Difference[2]			**	N.S.	*	**	**	**	**	**	N.S.

[1]Scoring System: 1 = 0-19%, 2 = 20-39%, 3 = 40-59%, 4 = 60-79%, and 5 = 80-100%.
[2]N.S. = not significant; * = S.D. at 5%, ** = S.D. at 1%.

less than 50%. The most southern seed source (Glades County, Florida) was consistently more severely infected than any of the other sources. Variation among physiographic provinces proved to be very small and nonsignificant when compared with the variation among sources within a province (table 2).

The correlations of infection with annual growth rate were nonsignificant for four plantations and highly significant in the other three plantations only if the Glades County source was included.

Table 2.--Analysis and variance components for brown spot needle blight of 20 seed sources of longleaf pine planted at seven locations in Georgia and Florida.

Sources of variation	Df	Mean squares[1]	Estimated variance components	Percent of total variation
Plantations	6	16.4279 **	0.1297	33.6
Province	5	1.4951 N.S.	0	0
Sources in Province	14	2.4359 **	.0546	14.2
Replications in Plantation	35	1.2791 **	.0601	15.6
Plantation X Province	30	.2413	0	0
Plantation X Sources in Province	79	.2544	.0228	5.9
Province X Replications in Plantations	175	.1197	.0006	.2
Error	465	.1178	.1178	30.5

[1]N.S. = not significant; ** = S.D. at 1%.

DISCUSSION

In spite of the lack of significant differences among the physiographic provinces, some useful and corroborating information is shown by the variation among the individual seed sources. The relatively high susceptibility of the seed source from Glades County, Florida, parallels the high level of infection reported for a seed source from Hillsborough County, Florida, planted in Mississippi in 1953 and 1954. Also, the average susceptibility of the lower Coastal Plain sources used in this study was similar to the results for the Treutlen County, Georgia, source in the same Mississippi plantings (Henry and Wells 1967).

The lack of a strong correlation between growth rate and infection supports the results of Snyder and Derr (1972), which indicated that fast height growth of longleaf pine was not a major factor in resistance to the brown spot fungus.

Although this study did not contain any of the known susceptible western sources (Henry and Wells 1967, Wells 1969), the Glades County, Florida source could serve as an indicator that the relatively low infection of the other sources had a genetic foundation and was not due to unusually low levels of inoculum at the planting locations. No single seed source possessed an exceptionally high resistance to infection by the brown spot fungus that could be utilized in a longleaf pine breeding or planting program similar to that for loblolly pine with resistance to fusiform rust, found in the seed source from Livingston Parish, Louisiana.

Instead these results indicate that the area of Georgia and north Florida could be a source of resistance, based on individual tree selection and progeny testing. Wakeley (1970) estimated that 100% gain in volume production at 30 years might be achieved by the development of an inherently resistant strain of longleaf pine.

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Control of Brown Spot Needle Blight Infection on Longleaf Pine Through Benomyl Treatment and Breeding[1]

Albert G. Kais and Margene M. Griggs[2]

Abstract.--A 2-year field study evaluated the relationship of brown spot needle blight infection to survival and growth of 29 longleaf pine (Pinus palustris Mill.) families varying in disease resistance and height growth. The study showed (1) a negative correlation between brown spot infection and either percent survival or height growth, (2) the effectiveness of a benomyl root dip treatment of seedlings for brown spot needle blight control, and (3) resistant longleaf pine families possess excellent early height growth even with high infection levels and no benomyl. Survival and growth rates were significantly increased by the use of resistant families combined with a benomyl fungicide root treatment.

INTRODUCTION

Longleaf pine (Pinus palustris Mill.) seedlings do not initiate height growth until they have passed through a preliminary period of root and foliar development referred to as the "grass stage" (Wahlenberg 1946). This condition is an inherent species trait under genetic control, although it is capable of being modified within limits by specific environmental factors (Brown 1964). Low soil fertility, competition from other plants, and infection by the brown spot needle blight fungus, Scirrhia acicola (Dearn.) Siggers, can delay the initiation of height growth up to 15 to 20 years (Bruce 1959, Dorman 1976). This has caused the formation of nonuniform stands that have been difficult to manage and harvest. Consequently, growers turned to other southern pine species for meeting production goals and quotas.

Brown spot needle blight has been a major factor inhibiting longleaf pine regeneration because it defoliates seedlings, reduces seedling vigor, and can cause mortality. Repeated defoliation, with subsequent reduction of plant vigor, delays the initiation of height growth. Once seedlings begin height growth, disease effects are minimal.

Recent studies have indicated that disease effects can be significantly reduced by planting resistant seedlings or by treating root systems of seedlings with the systemic fungicide benomyl (Benlate®)[3] [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate]. Inheritance of outstanding resistance in the F1 progeny of individual longleaf seedlings (Bey and Snyder 1978, Derr and Melder 1970) and increasing resistance through selection (Snyder and Derr 1972) suggest future disease control. With genetic manipulation, growth and survival have been increased (Snyder et al. 1977).

Although control of brown spot by fungicides is not practical for established longleaf plantings in the southern United States, benomyl root treatments have been extremely effective for control of disease on seedlings when applied prior to planting in the field (Kais 1981, Kais et al. 1981). Benomyl, applied either at lifting or planting, not only provided effective disease control, but also increased survival and growth of the seedlings.

This paper reports on the relationship of brown spot needle blight infection to survival and growth of 29 wind- and control-pollinated longleaf pine families that varied in disease resistance and in the initiation of height growth.

MATERIALS AND METHODS

Twenty-four control-pollinated and 5 wind-pollinated families were used (table 1).

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[1]Paper presented at the IUFRO Working Party Conference on Recent Research on Conifer Needle Diseases [Gulfport, Miss., U.S.A., October 14-18, 1984].

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[3]The mention of a company or trade name does not imply endorsement by the U.S. Department of Agriculture.

All but 1 of the 17 known parents that gave rise to the 29 families were either resistant or intermediate to brown spot infection. Half of the parents were also elite selections (best 10%) for outstanding height growth. The one parent, 12-13, was known to be very susceptible to brown spot infection and was also a poor grower. Abe is a disease-free longleaf seedling that was found growing in an abandoned nursery bed among other seedlings that were heavily infected. In April 1980, germinating seeds of each family were sown in trays measuring 10 by 30 cm. Ten seeds were sown per tray in a 2:1 (V:V) ratio of sandy loam to peat moss. Trays were maintained in the greenhouse under a 16-hour day length until March 1981, when they were transplanted into nursery beds at the Harrison Experimental Forest (HEF) at Saucier, Miss.

Seedlings were lifted from the nursery beds and outplanted at the HEF during January 1982. Seedlings of each family were divided into two groups just prior to planting. Roots of both groups were moistened in water; one group of seedlings was shaken in a plastic bag containing dry clay, while the other group was shaken in a bag containing a mixture of clay and benomyl (10.0% a.i. by volume). The field planting consisted of a split plot design based on fungicide treatment; the major plot was family in a randomized complete block design, with blocking based on the planting location. A Whitfield tree planter was used to plant seedlings 1 m apart in rows 3 m apart. Blocks were separated by a 3-m buffer zone.

Table 1.--Identity of 29 longleaf pine families.

Number	Family ♀ x ♂
1	3-25 X 22-216
2	3-356 X Abe
3	3-356 X 27-168
4	5-77 X 12-13
5	5-77 X wind
6	8-25 X 12-13
7	8-144 X 12-13
8	8-144 X 22-216
9	8-144 X 27-168
10	10-434 X 12-13
11	10-434 X 27-168
12	11-467 X 12-13
13	11-467 X Abe
14	11-467 X 27-168
15	13-286 X Abe
16	14-346 X 22-216
17	14-346 X 27-168
18	15-366 X 22-216
19	17-288 X 22-216
20	17-288 X 27-168
21	22-214 X 22-216
22	22-214 X Abe
23	22-216 X 12-13
24	22-216 X wind
25	27-168 X 22-216
26	27-168 X wind
27	30-142 X 12-13
28	30-142 X wind
29	Abe X wind

All seedlings were examined for percentage of brown spot infection, survival, and stem length during November 1982. Second-year evaluations were made of percentage of brown spot infection, survival, stem length, and percentage of seedlings in height growth in November 1983. Percentage of brown spot infection was determined following visual field estimates of the needle area affected by brown spot. Stem length was measured from the soil line to the tip of the terminal bud. Seedlings were considered to be in height growth when stem length exceeded 10.0 cm.

An analysis of variance (table 2) and Duncan's multiple range test were used to evaluate families and treatments. Correlation coefficients (r) were used to evaluate the relationship of brown spot infection to survival and to growth. In all cases, significance was evaluated at the 0.05 level.

Table 2.--Analysis of variance table.

Source	df
Blocks	4
Families	28
Error I	112
Treatment	1
Treatment X Family	28
Error II	116
Total	289

RESULTS

There was a significant benomyl X family interaction for each of the four variables, and these were due to scale effects. All families responded positively to the benomyl.

There was significantly less brown spot infection on the benomyl-treated seedlings than on the untreated seedlings after 1 year in the field (table 3). The untreated group had approximately seven times more infection. These infection percentages had a direct effect on both survival and stem lengths. The untreated seedlings had 11.4% less survival and approximately 10% less stem length growth than their benomyl-treated counterparts (table 3).

Table 3.--Effect of benomyl on brown spot needle blight infection, survival, and stem length of 29 longleaf pine families after 1 year in the field.

Plant response	Benomyl-treated	Untreated
Percent infection	9.4 (3.5-29.4)[1]	65.6 (33.7-85.8)
Percent survival	92.8 (68.8-100.0)	81.4 (46.7-100.0)
Stem length (cm)	6.5 (4.3-10.9)	6.0 (4.5-6.9)

[1]Range of means for the 29 longleaf pine families.

Although infection increased in the second year, the general pattern remained constant to the first year. Infection of benomyl-treated seedlings ranged from 6.4% to 38.1%, with an overall mean of 16.4%, while infection of the untreated seedlings had a range of 52.8% to 83.8%, with a mean of 67.6% (table 4). For each of the 29 families, the benomyl-treated seedlings again had significantly less infection than their paired counterparts. Significant differences in infection were evident for the two treatments of each family during both years of the study.

Untreated seedlings as a whole had approximately 15% less survival than the treated seedlings (table 4). Mean percent survival for the benomyl-treated group was 88.7%, while for the untreated group it was 73.9% (table 4). Although significant survival differences were evident among families, only 5 of the 29 families showed significant differences in survival between the paired treatments.

The benomyl-treated seedlings as a whole exhibited approximately twice the stem length growth as the untreated group (table 4). Mean stem length for the benomyl-treated group was 17.9 cm, while for the untreated group it was 9.3 cm. Significant differences in stem length were observed among families. Only 11 of the 29 families exhibited significant differences in stem length between the paired treatments.

For the percentage of trees in height growth, the benomyl-treated group averaged 50.5%, while the untreated group averaged 22.1%. There were significant family differences within each treatment group. Only 15 of the 29 families showed any significant difference in height growth between the paired treatments.

Correlations were made between percentage of brown spot infection and either survival or growth responses for the means of the 29 families after the first and second year in the field (table 5).

Table 4.--Brown spot needle blight infection, survival, and growth of 29 longleaf pine families after 2 years in the field.

Family ID No.	Response: Infection		Survival		Stem length		Height growth[1]	
	Benomyl	Clay	Benomyl	Clay	Benomyl	Clay	Benomyl	Clay
	----percent----		----percent----		-------cm------		----percent----	
1	19.9	72.6	74.4	55.9	12.0	5.9	34.0	10.0
2	15.5	52.8	97.1	94.3	18.3	11.4	57.8	26.5
3	17.5	61.3	95.0	92.1	23.7 *	12.1	68.7 *	28.1
4	10.4	77.1	84.3	67.9	9.0	5.9	12.4	9.0
5	10.7	72.3	86.1	64.6	8.9	6.5	32.0	20.7
6	28.4	80.3	93.3 *	46.7	7.3	4.2	10.5	5.0
7	12.5	84.9	91.8 *	46.8	9.6	5.4	25.8	3.3
8	10.7	61.9	86.3	74.1	10.6	5.4	41.7 *	6.9
9	7.9	60.1	100.0	94.6	24.1 *	10.7	73.2 *	30.8
10	18.8	68.1	81.7	81.3	9.1	7.6	26.6	19.2
11	11.9	64.4	96.7	86.0	38.7 *	15.7	70.7	52.0
12	13.8	75.2	88.6	77.5	14.5	7.6	43.5 *	15.7
13	13.4	68.7	94.6	90.6	24.8 *	10.5	71.7 *	36.2
14	6.4	53.5	95.0	93.3	37.6 *	15.5	82.3 *	47.9
15	29.2	69.1	96.7	86.4	12.5	10.3	39.3	23.3
16	12.6	59.2	80.0	82.5	12.1	9.0	36.2	20.0
17	8.7	65.6	97.5	89.3	18.3 *	7.3	63.9 *	15.7
18	14.9	56.2	88.9	67.6	21.3	13.6	62.6 *	23.3
19	22.1	62.4	82.5	69.3	26.6 *	11.7	83.6 *	31.9
20	7.9	58.4	97.1	86.8	26.3	19.4	67.5	57.7
21	18.6	66.8	85.2	83.7	21.2 *	5.5	69.9 *	8.3
22	7.8	53.2	96.0	81.3	15.4	14.5	46.3	50.0
23	38.1	83.9	59.9 *	21.7	10.7	5.1	38.7 *	0.0
24	21.9	71.2	79.5	67.8	16.3 *	5.5	50.3 *	6.9
25	16.7	70.0	92.5	100.0	26.6 *	12.0	77.5 *	27.9
26	13.0	57.7	93.8	76.9	23.3 *	11.9	77.0 *	32.3
27	25.8	76.7	80.5 *	52.8	10.3	7.5	22.7	18.3
28	16.4	88.3	85.0 *	33.3	13.6	5.4	29.2	0.0
29	24.3	68.1	92.1	75.0	15.0	6.8	48.9 *	14.4
Mean	16.4	67.6	88.7	73.9	17.9	9.3	50.5	22.1

[1]Percentage of seedlings exceeding 10.0 cm in stem length.
*Significantly different at the 0.05 level according to Duncan's multiple range test.

This was done for the benomyl-treated group and for the untreated group.

There were significant negative correlation coefficients (r) for all relationships tested, with one exception. There was no significant correlation between second-year infection of benomyl-treated seedlings and percentage of seedlings in height growth after the second year.

DISCUSSION

Using a benomyl root treatment for disease control proved to be a good method for evaluating the effect of brown spot infection on the survival and growth of brown spot resistant longleaf pine seedlings. For each family, infection was significantly reduced on the benomyl-treated seedlings as compared to its untreated counterpart (table 4). Survival, stem length, and percentage of seedlings in height growth were generally greater on the treated seedlings than on the untreated seedlings for each family.

Survival of the untreated seedlings could change significantly in the future. Third-year survival may be lower since some of the families already have greater than 60% infection. However, it is also possible that the resistant families in this test possess the capability to survive and grow with higher levels of brown spot infection (tolerance).

Stem length differences between the treated and untreated groups of a family increased after the second year's observations (tables 3 and 4). As with survival, height growth was generally good after 2 years, considering the severe infection. This was also probably due to the degree of resistance of the families used in the study.

Certain families appeared to be superior in their ability to survive and to begin height growth under the severe disease conditions (table 4). For all parameters, the five best performing families consisted of crosses with parent 27-168; these families were: 3, 9, 11, 14, and 20. Three of the five worst performing families consisted of crosses made with 12-13; these families were: 1, 6, 23, 27, and 28. This generally followed expectations since 27-168 is very resistant to brown spot and has excellent height growth, while 12-13 has been found to be quite susceptible and to have poor height growth (Snyder and Derr 1972).

All of the brown spot and growth response correlations over time were high, suggesting that early brown spot control and/or inherent resistance is very beneficial (table 5). Even though correlation values increased with time, it appears that first-year infection data may be used to predict future survival and growth trends. It was also possible to differentiate among families for resistance to brown spot needle blight even when using benomyl in field tests.

Table 5.--Correlation coefficient value (r) between family means for brown spot infection and survival and growth traits.

Treatment and percent infection	Response				
	1st year		2nd year		
	Survival	Stem length	Survival	Stem length	Height growth[1]
Untreated progeny					
Percent brown spot 1st year	-0.79[2]	-0.46	-0.72	-0.63	-0.70
Percent brown spot 2nd year			-0.75	-0.72	-0.72
Benomyl-treated progeny					
Percent brown spot 1st year	-0.57	-0.38	-0.51	-0.44	-0.45
Percent brown spot 2nd year			-0.56	-0.38	-0.32

[1]Percentage of seedlings exceeding 10.0 cm in length.
[2]Values exceeding ±0.36 indicate significance at the 0.05 level.

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A Needle Blight of Slash and Loblolly Pines in South Mississippi[1]

G. A. Snow[2]

Abstract.--A severe needle blight of Pinus elliottii (Engelm.) and P. taeda L. occurred over 53 million acres of southern pine forests during the late fall and winter of 1970-71. Symptoms first appeared in late November and December; but signs of fungi were not found on the needles until February and March of 1971, when Lophodermella cerina Darker, Ploioderma lethale (Dearn.) Darker, and P. hedgcockii (Dearn.) Darker were found on needle collections from several southern states. These fungi were presumed to have been the causal agent. Although the disease has recurred each year since 1970 in southern Mississippi, incidence and severity have been much less than observed in 1970-71.

INTRODUCTION

A severe needle disease was reported by foresters in Hancock County, Mississippi, in early December of 1970. A dense fog had obscured the area from November 27 to December 2. When the fog lifted, foliage of slash and loblolly pines (Pinus elliottii Engelm. and P. taeda L.) was observed to have turned reddish brown. Almost all of the trees in the county were affected. Soon thereafter, similar reports came in from foresters in several southern states. An aerial survey was made of the southern pine region in January 1971 (Wolfe et al. 1971), and 53,852,440 acres were found to be affected in the southern portions of seven states (fig. 1). The widespread disease of unknown cause resulted in considerable concern to foresters and researchers alike.

Slash pines were affected more than loblolly pines, and longleaf pines (P. palustris Mill.) appeared immune. The disease was most severe on large trees. Although trees were affected uniformly throughout their crowns, there was much variation in the amount of needle damage among trees. On some trees only the needle tips were damaged, while on others, over two-thirds of the needle area was brown.

Needles formed in both 1969 and 1970 were affected. The distal portion of the needles was reddish brown, with a dark brown demarcation between the dead area and the green base of the needles. The appearance of these needles did not change until March and April 1971, when they became completely brown and fell from the trees.

No needlecast fungi were found on the needles until late February and March of 1971. When fruiting structures did form, needle collections were examined from all states where the blight was observed (Czabator et al. 1971). The most prevalent pathogenic fungi identified were Lophodermella cerina Darker, Ploioderma lethale (Dearn.) Darker, and P. hedgcockii (Dearn.) Darker. These fungi were thought to have caused the disease, but the reason for the widespread epidemic was not determined.

Boyce (1954) described a similar needle blight caused by P. lethale, which occurred from 1949 to 1952 on hard pines in the Atlantic States. The symptoms he described, however, consistently appeared in March and April--3 to 4 months later than those observed in the southern states in 1970. Whether these differences in time of symptom expression were due to differences in the fungi involved or to the conditions under which the diseases developed is unknown.

Since 1970, the blight has recurred each year in November or December in southern Mississippi, but its magnitude has been much less than in 1970-71. This suggests that adequate inoculum is present every year, but the sequence of weather conditions that results in severe infection and

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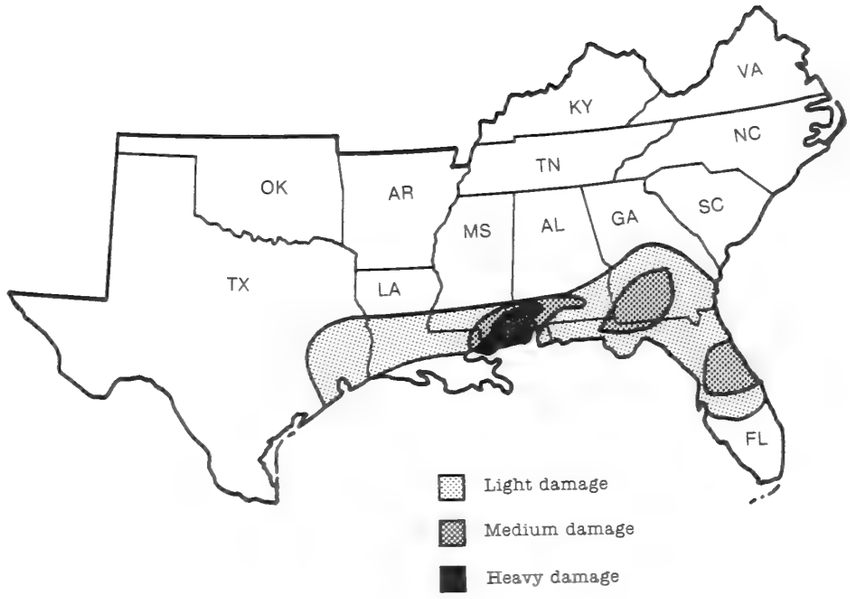

Figure 1.--The approximate distributions and intensity of needle blight throughout the South, March 1971. From Wolfe et al. 1971.

severe symptom expressions are rare. In 1970, spring and summer were near normal along the Mississippi coast, except that precipitation in April was below average. The autumn was unusual--temperatures in November were well below normal; and a hard freeze (-6° C) occurred on November 25 and 26, followed by several periods of dense fog, each lasting for 4 to 7 days. The hard freeze is now thought to have been an important factor in the 1970 epidemic because every year since 1970, the disease symptoms have consistently appeared within 2 to 3 days after the first killing frost or the first period of freezing weather.

The zone of heavy damage from the blight, extending from near Slidell, La., across southern Mississippi to north of Mobile, Ala. (fig. 1), suggests that atmospheric deposition from the metropolitan areas of New Orleans and the Gulf Coast could have magnified the severity of the disease. An oil well located near the mouth of the Mississippi River in the Gulf of Mexico caught fire during the latter part of November and burned until early December 1970. The temperature inversions associated with the dense fogs in 1970 would have concentrated and held whatever air pollutants were present at the time. Therefore air pollution cannot be excluded as a contributing factor.

Apparently, this needle blight of slash and loblolly pine does not kill the trees, and the only damage is a slight reduction in growth. The disease is therefore not recognized as a significant problem in southern forests.

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Infection Processes of Three *Lophodermium* Species on *Pinus sylvestris* L.[1]

S. A. Diwani and C. S. Millar[2]

Abstract.--Infection processes of Lophodermium pinastri, L. conigenum, and L. seditiosum have been studied. On current-year needles of transplanted Pinus sylvestris, all three Lophodermium species penetrated the host directly through the cuticle and rarely through stomata. L. seditiosum was the only species found to be capable of colonizing green needles of the transplants. Colonization of tissues was by "hyphal complexes" which are thought to be important structures in latency exhibited by the Lophodermia.

INTRODUCTION

In the conclusions of our last meeting, the Working Party emphasized the need to do detailed studies on the infection processes of the most important needle diseases so as to be better equipped in the studies of their cultural and biological control methods. Needle-cast disease of Pinus species caused by Lophodermium seditiosum Minter, Staley & Millar in close association with L. conigenum Hilitzer and L. pinastri (Schrad.) Chev., is widespread and a well-recognised needle disease in nurseries in Europe and North America. Consequently, a considerable amount of work on breeding for resistance and chemical control methods for the disease has been done, but surprisingly little has been published on the actual processes of infection.

Before the publication of Lophodermium seditiosum (Minter et al. 1978), previous literature on the infection process of L. pinastri has inevitably included data on observations from more than one species. This has caused confusion in the literature. In this paper, whenever information is thought to have included data from more than one or the wrong species, the genus and the species are enclosed in quotation marks (e.g. 'L. pinastri').

Jones (1935), the earliest worker to report on the process of infection of P. sylvestris by 'L. pinastri', observed that infection was through stomata. Without demonstrating independent observations, Lanier (1968) indicated support for Jones. Disputing Jones' conclusion, Costonis et al. (1970) postulated that 'L. pinastri', gains ingress into P. sylvestris and P. strobus by penetrating directly through cuticle. This is in accord with Rack (1959), who made the point that there was no site relation between infection spots due to 'L. pinastri' and the stomata.

The most convincing study on the initial stages of infection of P. sylvestris by L. seditiosum was made by Staley (1975). He observed that L. seditiosum penetrated its host directly through the cuticle. Like Costonis et al, Staley noted appressorium formation by the germinating ascospores. Under natural conditions, formation of an appressorium was said to be the main method of germination (Staley 1975). The most obvious difference in the reports was that Costonis et al reported penetration and advanced stages of tissue colonization within 3 weeks after ascospore deposition on the needle surface, but Staley judged that penetration must take at least 4 to 5 weeks after ascospore deposition on the needle surface. An interesting aspect, reported by Staley, was that penetrating hyphae grew directly through the cuticle from melanized appressoria. He commented that appressorial melanization may be a necessary process for penetration and that melanized appressoria may be resistant to fungicides.

Differences in the described infection process clearly need clarification. In an attempt to disentangle the confusion, infection processes of three Lophodermium species (L. pinastri, L. conigenum, and L. seditiosum) have been studied on a single host (Pinus sylvestris).

MATERIALS AND METHODS

Inoculations were made on 14-month-old P. sylvestris transplants in John Innes No. 2 potting compost in 12.5 cm cuboid pots. There were 10

[1]Paper presented at the IUFRO Working Party Conference on Recent Research on Conifer Needle Diseases [Gulfport, Miss., U.S.A., October 14-18, 1984].

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plants in each of 20 pots. Each Lophodermium species was inoculated, at the time of its peak of natural sporulation, on plants in five pots. The remaining five pots were used as uninoculated controls. For inoculation, 100 needles, bearing naturally produced ascocarps and selected carefully so as not to mix species, were first soaked until the ascocarps opened and then laid on the 10 transplants in each pot. Inoculations were started on 4 July 1980 for L. pinastri; 4 October 1980 for L. seditiosum and 4 November 1980 for L. conigenum. Each inoculated and control pot was enclosed in a polythene bag for 2 weeks to encourage accumulation of moisture, which was found to be necessary for the continued release and germination of ascospores. During and after inoculation, the pots were kept outdoors under natural fluctuations of light and temperature, to simulate as nearly as possible field conditions.

For observations of the germination processes and symptom development, needles were first detached 7 days after the start of inoculation and subsequently at approximately 2-week intervals for 14 weeks. The nail varnish technique (Delp 1954) was used to remove ascospores, after staining them with basic fuchsin (Preece 1959), for observation of germination and appressorium formation. Penetration and colonization were observed by sectioning pieces of the needles, which bore stained ascospores, infiltrated with 1.5% aqueous Reten using a freezing microtome. The sections were mounted in 1% cotton blue in lactophenol and observed under the light microscope. Additional needles were examined in the scanning electron microscope.

In order to study in more detail the timing of the early stages of infection, observations were made also on inoculated detached needles incubated at 100% RH and 20°C in glass dishes in the laboratory. The needles were inoculated with ascospores from selected ascocarps suspended for 8 hours over the detached needles. Needles were removed daily for the first 5 days and then at 5-day intervals for up to 14 weeks. Germinating spores were stained with basic fuchsin and observed directly with the light microscope to avoid any artifacts due to the nail varnish technique. Observations of penetration and colonization were made as above.

RESULTS

Prepenetration.--After ascospore deposition swelling of the mucilage sheath covering the ascospores occurred, resulting in an increase of diameter from 2.5 to 8.0 µm. After mucilage swelling many vacuoles developed in the ascospores and a central septum, which divided 98% of L. conigenum and L. seditiosum ascospores, was formed. Septa were not common in L. pinastri ascospores. All these changes occurred within 8 hours after ascospore deposition.

Observations made 12 hours after ascospore deposition revealed that germination structures of all three species had started to form. For all three species, maximum percentage germination was achieved between 36 and 48 hours. Mean percentage germination at 20°C was higher for L. seditiosum (78%) and L. conigenum (77.4%) than for L. pinastri (22.7%).

There were five types of germination (fig. 1). In type I the ascospores formed a long, thin germ tube, but in type II a short, thick germ tube, which terminated its growth by the formation of an appressorium, was formed. Type III was by the direct formation of an appressorium. Types I, II, and III were shown by each species. Type IV was shown only by L. pinastri. In type IV the ascospore germinated by swelling at any point along the spore. On nutrient media (e.g. 2% malt extract agar) this type of germination often led to ascospore disintegration as observed by Schütt (1960, 1967) and Lanier (1968). Type Va was exhibited when ascospores of L. conigenum and L. seditiosum were germinated on an inert petri-dish surface where no nutrients were available. In one of his illustrations of germinating ascospores of 'L. pinastri', Darker (1932) shows a germination type similar to this where an apparent hyphal complex is formed on germination (fig. 1, type Vb).

During germination cytoplasmic material, which stained deep blue with 1% cotton blue in lactophenol, from the ascospore was observed to have moved inside the germinating structures into the appressoria or germ tubes. The appressoria, most of which were formed in the grooves on the needle between the epidermal cells, expanded sufficiently to accommodate the cytoplasmic material. At this stage the ascospores appeared empty. After appressorial expansion was over, a septum separating the appressorium from the main ascospore body was formed. By using a scanning electron microscope, it was observed that at this stage the mucilage sheath had collapsed (fig. 2). With germination type I most of the germ tubes elongated further with the formation of more septa in the germ tube, but no appressoria were formed. With type IV movement of cytoplasmic material was not observed.

Expansion of the appressoria took about 1 week after the first germination structures had been observed, and at this stage the process of appressorial wall thickening appeared to have started. Wall thickening and melanization were not obvious with germination type IV. Observations made 1 week after the wall thickening had started revealed that melanization of the appressorial wall was taking place. In week 3 after ascospore deposition, vacuoles were observed to have formed in the appressorium. The vacuoles disappeared in week 4 after ascospore deposition leaving a light-colored circular mark at the bottom of the appressorium. The circular mark is believed to have been the point of origin of the infection peg.

Penetration.--Sectioning of needles bearing melanized appressoria revealed that all three Lophodermium species penetrated the host directly through the cuticle. Penetration occurred in week 4 after deposition of the ascospores. Occasionally, thin hyphae were observed to have penentrated through stomata and signs of substomatal vesicle formation were observed but stomatal penetration was extremely rare (i.e., ca.1%).

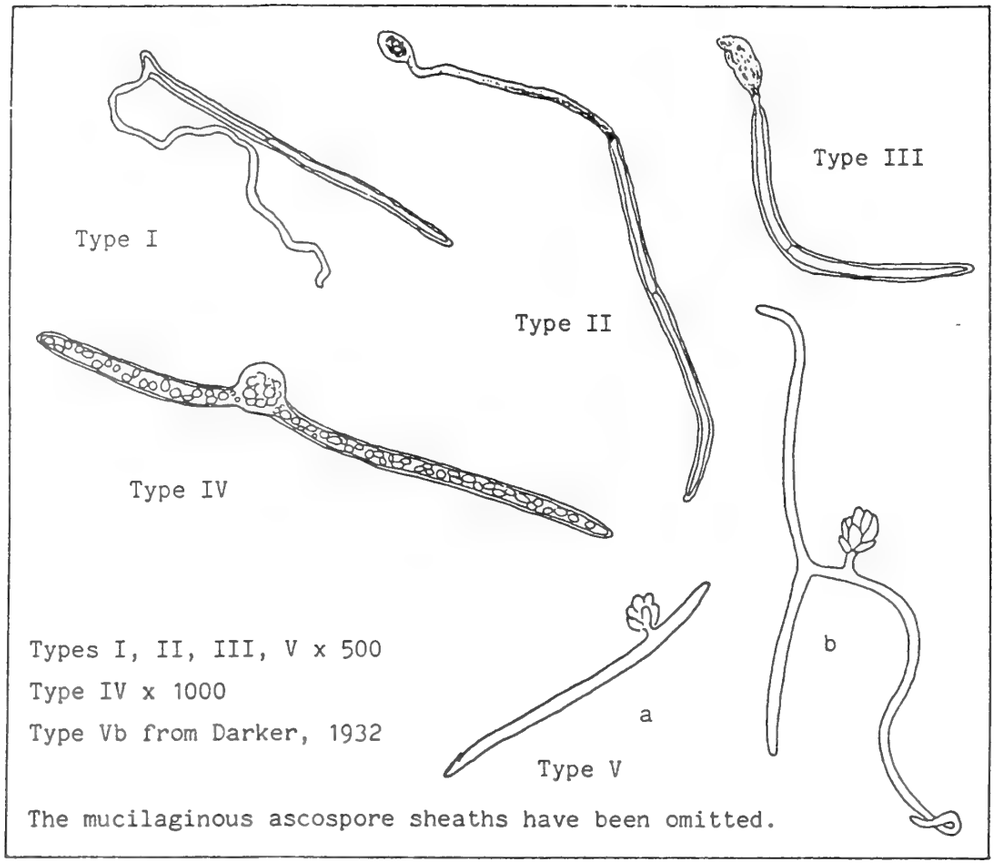


Figure 1. Lophodermium ascospore germination types.

Postpenetration.--Immediately after penetration differences between species were observed. With L. pinastri, the infecting hyphae were observed to form "bladderlike" hyphae in the cuticles but necrosis of the cells below was not observed (fig. 3). At this stage sectioning of needles inoculated with L. pinastri revealed no further growth of the fungus from the cuticular region and no lesions were formed. Remnants of L. pinastri ascospores on the needle surface could not be located in week 14 after ascospore deposition and, as no microsymptoms of infection were visible, this made further sectioning of needles a fruitless task. However, observations of 2- to 3-mm diameter infection spots, on 2nd and 3rd year needles from 16-year-old trees, revealed that L. pinastri could be isolated from them, suggesting that further colonization of aging tissues by L. pinastri, resulting in the formation of the infection spots, does take place but is not extensive enough to cause macrosymptoms.

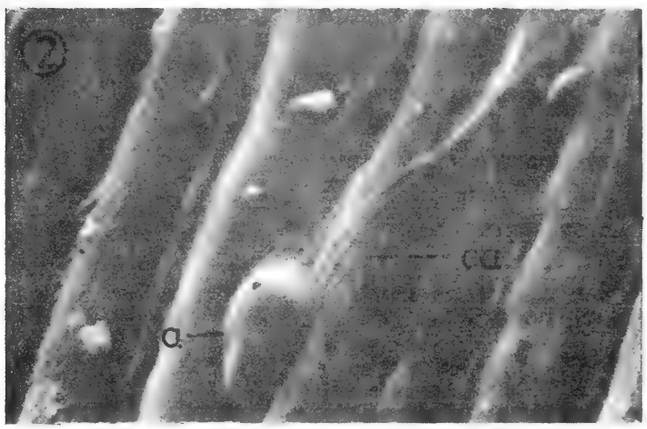
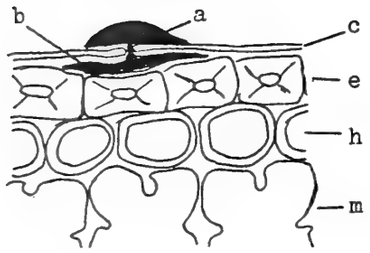


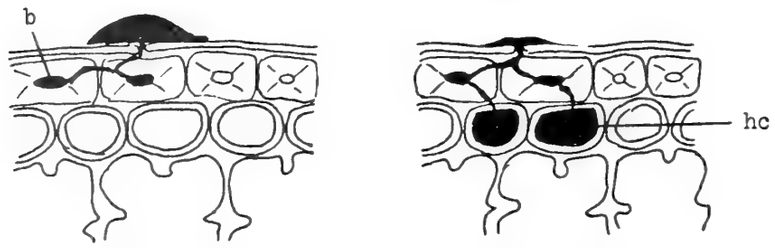
Figure 2. Collapsed L. pinastri ascospore (ca) with an appressorium (a) on needle surface of Pinus sylvestris (x 1000).

With L. conigenum intracuticular hyphae were rare but, in most cases, bladderlike hyphae grew in the epidermal cells and further into the hypodermal cells through the plasmodesmata and formed "hyphal complexes" in the cell lumen (fig. 3). The complexes which are similar to those formed in germination type V became melanized both when formed on an inert surface and in the plant cells, which made it difficult to stain them. This may be the reason why Hartig (1882) found it difficult to detect the presence of Lophodermium in the early stages after the death of needles killed by Lophodermium. After the formation of complexes in the hypodermal cells no further growth of hyphae was observed. As with L. pinastri, since ascospore remnants and microsymptoms could not be observed 12 weeks after ascospore deposition, further sectioning of needles was discontinued. Again, on 2nd to 3rd year needles from 16-year-old trees, L. conigenum could be isolated from brown infection spots with yellow margins but no macrosymptoms developed unless the branches or trees were damaged.



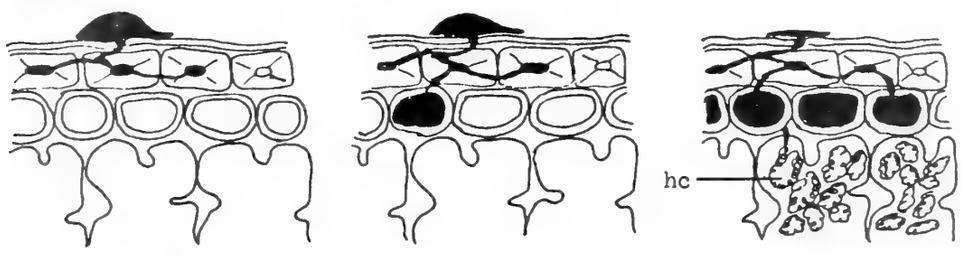
L.pinastri.

Colonisation depends upon needle senescence or damage.



L.conigenum. 12 weeks

Colonisation depends upon weakening or death of the tree, branch or shoot.



L.seditiosum. 10 weeks 12 weeks

Colonisation is progressive into the stele and the needle dies.

Figure 3. Postpenetration differences between Lophodermium infections. a = appressorium, b = bladder hyphae, hc = hyphal complex, c = cuticle, e = epidermis, h = hypodermis, m = mesophyll.

With L. seditiosum, the infecting hyphae first formed bladderlike hyphae in a single epidermal cell and then colonized the adjacent epidermal cells. Under a microscope yellow spots could be observed in the same week that penetration had occurred. These microsymptoms could be used to distinguish which ascospore had infected and which one had not. The microsymptoms were composed of about 3-5 epidermal cells transversely and about two cells longitudinally. Where infecting ascospores were numerous, initial symptoms appeared as yellow flecks. These yellow spots or flecks could be observed by the unaided eye 7 weeks after inoculation but were very temporary and had turned brown a week later. At this stage, colonization was mainly of epidermal cells, with only a few hypodermal cells affected (figs. 3 and 4).

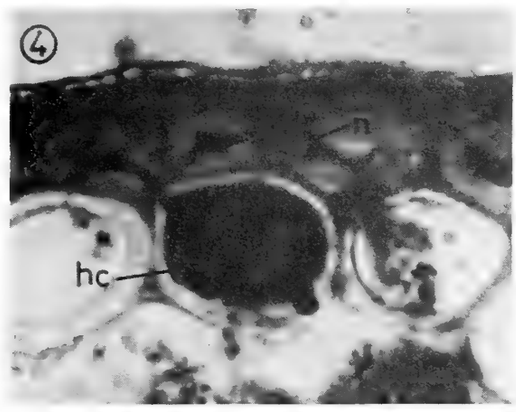


Figure 4. L. seditiosum normal hypha (n) in an epidermal cell and a 'hyphal complex' (hc) in a hypodermal cell (x 1250).

In week 10 after inoculation, large numbers of epidermal cells and some hypodermal cells had been colonized. Infection spots or flecks were already brown and their expansion was marked by further browning.

Colonization in the hypodermal cells was by hyphal complexes similar to those described for L. conigenum. In week 12 after inoculation, hyphal complexes had penetrated the mesophyll cells both intracellularly and intercellularly in the disorganized tissues (fig. 4). Disorganization of tissues always preceded colonization of the cells by the hyphal complexes. Penetration of the stelar tissues occurred 16 weeks after inoculation. By this time, individual infection spots or flecks had expanded up to 5 mm along the needles. Heavily infected needles turned brown in most areas. Death of needles occurred 3 weeks later, so most of the needles were killed 5-6 months after inoculation.

Sectioning of killed needles revealed that further colonization of dead tissues was not by hyphal complexes but by the ordinary type of hyphae observed in normal cultures.

DISCUSSION AND CONCLUSIONS

The method of penetration of the host tissues by all three species was by direct penetration of the cuticle by an infection hypha from an appressorium. Workers who observed regular penetration through stomata were perhaps dealing with Cyclaneusma minus Di Cosmo, Peredo & Minter (Naemacyclus minor Butin), which is a close associate of L. seditiosum in nurseries and which penetrates through stomata (Karadžić 1981). Because penetration through the cuticle reduces the dependence of infection processes on stomatal opening or wounding, Lophodermium species are well adapted as conifer needle pathogens.

Staley (1978) pointed out that, since fungicides largely prevented both appressorial melanization of L. seditiosum and symptom development it appears that appressorial melanization is an essential step in the process of leaf penetration by L. seditiosum. Our observation, that penetration occurred in the 4th week after appressorial wall thickening and melanization, supports Staley's speculation. Examination of thin needle sections at regular intervals after inoculation showed that penetration occurred only after appressorial wall thickening and melanization. Thus, studies on host resistance factors that hinder appressorial development or melanization may possibly offer opportunities for a method of biological control of the disease.

On transplant needles, L. seditiosum was observed to colonize green needles by hyphal complexes. These were not formed on artificial nutrient media or in dead needles with no host resistance. However, on an inert petri dish surface, where no nutrients were available, complexes did form. This indicates that the complexes are formed when the fungus is under stress, implying that, in live needles, there are chemical factors of resistance that force the advancing hyphae to form complexes to resist these factors. From this, it appears that studies to investigate the chemical rather than the physical factors of resistance are most likely to lead to discoveries that may simplify the selection of resistant trees. Already some advances have been made with studies on needle extracts (Schütt 1964) and buffer capacity (Stephan and Scholz 1981).

Millar (1981) suggested that the means by which the fungi can remain dormant in infected needles should be investigated together with the physiological factors that predispose needles to colonization from latent infections, since many diseases might be prevented by improved culture conditions. With L. seditiosum the formation of hyphal complexes was the means by which the fungus colonized its host. The colonization process was slow but L. seditiosum does not really stay latent. However, with L. conigenum we believe that hyphal complexes may be the means by which the fungus stays latent until the branches are damaged. L. pinastri was not observed to form complexes in transplant needle cells but appears to stay latent in the cuticle. However, we believe that similar structures may be formed by L. pinastri in older needles and that young transplant needles may not have been the best material for studying colonization by L. pinastri.

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Genetic Variation of Resistance to *Lophodermium* Needle Cast in Scots Pine Progenies of Intraprovenance and Interprovenance Crossings[1]

B. R. Stephan and D. Krusche[2]

Abstract.--The Lophodermium needle cast resistance of 96 families of crosses between phenotypically selected Scots pine trees (Pinus sylvestris) of a Mazurian and an Alpine provenance was investigated at two locations in northern Germany. The fungal attack was evaluated on 9-year-old crossings and showed highly significant genetic variation between and within the intraprovenance and interprovenance crossings. A quantitative genetic analysis of the data showed strong additive genetic variation and a relatively high heritability of resistance. Resistance to Lophodermium seditiosum might be incorporated successfully into breeding programs in Scots pine.

INTRODUCTION

The needle cast disease caused by the fungus Lophodermium seditiosum Minter, Staley & Millar, is the most serious foliage disease of Scots pine (Pinus sylvestris L.) in the temperate zone. In some years heavy infections can cause severe defoliation to young pine stands over large areas and a reduction of tree growth. Chemical control of this economically important disease is expensive and problematic for reasons of environmental protection. Therefore, the search for resistant or less susceptible populations or individuals is of great significance. Results of past investigations are summarized in various papers (Schütt 1958/59, Lanier 1969, Stephan 1975a, Stephan and Scholz 1975, Martinsson 1979), which concluded that significant differences of susceptibility to Lophodermium needle cast exist between Scots pine populations and between single trees even within susceptible populations. Only a few data are available about the genetics of needle cast resistance (e.g., Squillance et al. 1975, Johnsson 1976, Martinsson 1979). Resistance is probably polygenically inherited (Langner 1952, Hattemer 1965), but further studies are needed to confirm this hypothesis.

In this paper, results of needle cast attack in Scots pine progenies of intraprovenance and interprovenance crossings are presented. Crossings were made for the purpose of studying possible heterosis effects. First results on height growth, needle cast disease, and buffering capacity of needle extracts were published earlier for some of the crossings (Stephan and Scholz 1980, 1981). This paper presents a more detailed evaluation of the response of the total crossings to the Lophodermium needle cast attack on two locations in northern Germany in 1983.

MATERIALS AND METHODS

Origin and Selection of the Scots Pine Clones.--Ten Mazurian single trees (origin Poland and U.S.S.R.) were selected on the basis of phenotypic characteristics (performance, form, etc.) in two Scots pine provenance trials near Rendsburg (Re) and Neumünster (Neu) in Schleswig-Holstein (northern Germany). Seven Alpine plus trees (Fö) were selected in two neighboring populations in Switzerland (table 1). Pollen was collected from another Alpine plus tree from Berchtesgaden (Ber) in a north German provenance trial. The provenance data are given in Table 1. Resistance to needle cast disease was no criterion for the phenotypic selection of these Scots pine clones. In this study the Alpine and the Mazurian group of Scots pine parents will be distinguished. Scions of the plus trees had been grafted on Pinus mugo rootstocks in the nursery of the Institute in 1951-53. The clonal material had been inoculated artificially with Lophodermium between 1956 and 1958 (Schütt, unpublished).

[1]Paper presented at IUFRO Working Party Conference on Recent Research on Conifer Needle Diseases [Gulfport, Miss., U.S.A., October 14-18, 1984].

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Table 1.--Origin of the Scots pine clones.

Parent clones	Origin	Latitude (°N)	Longitude (°E)	Altitude (m)
Mazurian clones				
Re 1, Re 2, Re 3, Re 6, Re 7, Re 8, Re 9, Re 10	Trappönen, East Prussia (U.S.S.R.)	55°02'	22°25'	
Neu 19, Neu 22	Grünheide, East Prussia (Poland)	54°51'	22°00'	200
Alpine clones				
Fö 41, Fö 42, Fö 45	Wassen (Switzerland)	46°46'	8°06'	1000
Fö 51, Fö 52, Fö 53, Fö 54	Intschi (Switzerland)			700
Ber	Berchtesgaden (Fed. Rep. of Germany)	47°06'	13°00'	1000

Controlled Pollination and Mating Design.--During 1965 and 1970 the clones had been crossed according to a factorial mating design with 17 female and 6 male parents; the crossings resulted in 96 different progenies (table 2). Four main mating groups can be distinguished: two intraprovenance and two interprovenance mating groups.

Progeny Test.--Seeds were sown in the nursery in 1974 and the seedlings of 96 families were transplanted in 1975. In 1977, these seedlings were outplanted in two field trials in northern Germany: Grosshansdorf (53°39' N., 10°12' E., 40 m altitude) and Syke (52°55' N., 8°32' E., 43 m altitude). The seedlings were planted in a completely randomized blocks design with four replications in square plots: in Grosshansdorf, 20 families with 16 plants/plot and 76 families with 4 plants/plot; in Syke, 76 families with 16 plants/plot.

Needle Cast Attack.--The attack by Lophodermium was scored in the individual trees in the spring of various years, but for the present study only the attack of spring 1983 was considered. Each tree was assigned a single score based on the following scale:

0 No infection.
1 Weak infection; few brown needles.
2 Moderate infection; 10-50% brown needles.
3 Moderate to heavy infection; 50-100% brown needles.
4 Very heavy infection; all needles brown or dropped.

Statistical Methods.--The basic units of observation for the analysis of variance are the plot means of Lophodermium attack, weighted with the number of surviving trees per plot (year 1983). The calculations of the variance components were based on the unweighted plot means. The within plot component was estimated separately from the individual disease scores within plots. For the statistical evaluation the software package GLIM (General Linear Interactive Modelling) was used (Baker and Nelder 1978).

RESULTS

Variation of Needle Cast Resistance Between Progenies

All progenies showed needle cast damage to a certain degree; an absolute resistance or immunity could not be observed, among the progenies. Nevertheless, the comparison of the mean scores for needle cast attack shows clear and considerable phenotypical differences between the Scots pine progenies (table 2). The mean attack varies from 1.5 to 4.0 on the two locations with a total average of 3.14.

Compared with the previous years the needle cast attack was strongest in 1983, the year consequently used for this study. The ranks of the progenies analyzed in an earlier investigation (Stephan and Scholz 1981) were strongly correlated with the 1983 data (correlation with year 1978 r = 0.96, with year 1980 r = 0.90).

There were also differences in needle cast attack scores between the two locations (table 2). The trees were less infected in Grosshansdorf (mean score 2.0) than in Syke (mean score 3.2). If we compare the needle cast scores of the 76 progenies planted on both locations, we find strongly correlated reactions (r = 0.81).

The mean mortality of the Scots pine trials until 1983 was relatively low, 16% in Grosshansdorf and 13% in Syke. There was a good positive correlation between the mean score of a family and the number of killed trees, showing that the more susceptible progenies had the higher mortality after 6 years under field conditions.

Variation of Needle Cast Resistance Between Mating Groups and Between Parents

The Scots pine progenies can be separated into four mating groups, the two intraprovenance groups

with the offspring of the Alpine and the Mazurian parents, respectively, and the two interprovenance groups with crossings between Alpine female and Mazurian male parents and vice versa. In table 2 differences between the mating groups can be recognized. The significance of these differences was confirmed in an analysis of variance (table 3), which showed additionally significant site effects and interaction between mating groups and sites. Obviously the reactions of the mating groups on the two locations did not correspond completely. The environmental factors responsible are unknown.

Table 2.--Factorial crossing design between Alpine and Mazurian Scots pine clones. Mean scores of Lophodermium attack (1983) of the progenies on two locations (upper figure for trial Grosshansdorf, lower figure for trial Syke).

♀ / ♂→	Alpine clones			Mazurian clones		
	Ber	Fö 52	Fö 53	Re 2	Re 3	Re 7
Fö 41	2.2	4.0	3.5	3.0	2.7	2.9
	3.7	4.0	4.0	3.8	3.7	3.8
Fö 42	2.6	3.9	3.3	2.2	2.2	2.8
	3.6	4.0	3.8	3.5	3.5	3.4
Fö 45	1.9	---	3.5	2.4	2.2	2.2
	2.7	---	3.8	3.0	3.3	3.3
Fö 51	3.4	4.0	4.0	3.7	3.5	3.9
	3.8	4.0	4.0	4.0	3.9	4.0
Fö 52	3.5	---	3.9	2.9	2.5	3.2
	3.7	---	---	---	---	---
Fö 53	2.5	3.7	---	2.5	2.1	2.8
	3.9	---	---	---	---	---
Fö 54	2.8	3.9	4.0	3.3	2.7	2.9
	3.8	4.0	4.0	3.6	3.7	3.7
Re 1	2.6	3.6	2.7	2.1	2.4	2.7
	2.6	3.9	3.8	3.1	3.6	3.3
Re 2	2.1	3.1	2.6	---	1.8	2.1
	2.5	---	---	---	---	---
Re 3	1.8	2.6	2.2	2.0	---	1.8
	3.2	---	---	---	---	---
Re 6	2.3	3.6	3.7	2.1	2.1	3.5
	3.0	4.0	3.9	3.0	3.2	3.9
Re 7	1.9	3.2	2.6	1.5	2.0	---
	2.7	---	---	---	---	---
Re 8	2.5	3.7	3.6	2.3	2.6	2.9
	3.5	3.9	3.9	3.0	3.1	3.9
Re 9	2.1	3.4	2.8	2.1	2.7	2.4
	3.3	3.9	3.8	3.2	3.6	3.4
Re 10	2.1	3.4	3.0	2.0	2.0	2.3
	2.5	3.8	3.7	2.8	3.1	3.3
Neu 19	1.9	2.7	2.7	2.2	2.0	1.9
	2.4	3.3	3.6	2.6	3.1	3.3
Neu 22	1.9	2.5	2.4	2.0	1.8	2.2
	2.5	3.1	3.6	2.8	3.2	3.2

Table 3.--Analysis of variance of infection score of different female and male parents on two locations.

Source	df	MQ	F-value[1]
Site (s)	1	945	359.3***
Mating group (g)	3	129.7	49.3***
s x g	3	34	12.9***
Between plots	667	2.63	---
Site (s)	1	945	393.8***
Female (f)	16	38.4	16.0***
s x f	16	3.9	1.6 n.s.
Between plots	641	2.4	---
Site (s)	1	945	378.0***
Male (m)	5	101.2	40.5***
s x m	5	10.4	4.2**
Between plots	663	2.5	---

[1]Significance: ** ≙ $p \leq 1\%$, *** ≙ $p \leq 0.1\%$, n.s. ≙ $p > 5\%$.

Significant differences in the degree of needle cast attack existed not only between the mating groups, but also between the progenies on the basis of all female and male parents (table 3). Interactions with the locations were found only for the male parents.

On the basis of these results, however, the progenies of crossings between the Mazurian parents were generally less severely attacked by the needle cast disease than the progenies of crossings between the Alpine parents (fig. 1; table 4, last line). The progenies of interprovenance crossings were attacked intermediately.

Variation of Needle Cast Resistance Within Mating Groups

The needle cast attack of the crossings differed not only between the mating groups, but also significantly within them. The results of an analysis of variance on the basis of female and male parents (table 5) can be interpreted to show that the variation between the progenies within a mating group was influenced very strongly by the female and male parents used for the crossings. The total means of the progenies with a distinct clone as female or male parent vary within the respective mating group (table 4). But the ranks of the female or male parents correspond very well in the single mating group and in the overall average for all mating groups, as is visible also in figure 2.

Although the reaction of the original parent trees to the Lophodermium needle cast disease was not known, the relative behavior of the artificially inoculated clonal material could be surmised. According to the results of the very heavy infections Schütt (unpublished data) surmised

Table 4.--Weighted mean infection score and rank of female and male parents, differentiated by mating groups. (M = Mazurian, A = Alpine)

Clone no.	M x M	Rank	A x A	Rank	M x A	Rank	A x M	Rank	All mating groups	Rank
Female										
Fö 41			3.63	3			3.58	5	3.60	5
Fö 42			3.70	4			3.26	3	3.46	4
Fö 45			3.11	1			3.01	2	3.05	2
Fö 51			3.81	6			3.87	7	3.85	7
Fö 52			3.73	5			3.85	6	3.21	3
Fö 53			3.62	2			2.47	1	2.93	1
Fö 54			3.83	7			3.55	4	3.67	6
Re 1	3.14	7			3.36	7			3.25	7
Re 2	1.94	3			2.64	2			2.36	2
Re 3	1.91	2			2.62	1			2.35	1
Re 6	3.22	9			3.53	9			3.38	9
Re 7	1.77	1			2.76	3			2.39	3
Re 8	3.15	8			3.67	10			3.41	10
Re 9	3.22	9			3.50	8			3.36	8
Re 10	2.85	6			3.22	6			3.03	6
Neu 19	2.79	4			3.01	5			2.89	5
Neu 22	2.83	5			2.93	4			2.88	4
LSD (5%)	0.45		0.36		0.34		0.34		0.28	
Male										
Ber			3.41	1			3.22	2	2.98	1
Fö 52			3.90	3			3.13	1	3.55	3
Fö 53			3.85	2			3.34	3	3.49	2
Re 2	2.61	1			2.70	1			2.85	1
Re 3	2.85	2			3.46	3			2.97	2
Re 7	3.03	3			3.34	2			3.16	3
LSD (5%)	0.25		0.28		0.19		0.25		0.19	
Mean score	2.82		3.63		3.15		3.23		3.14	

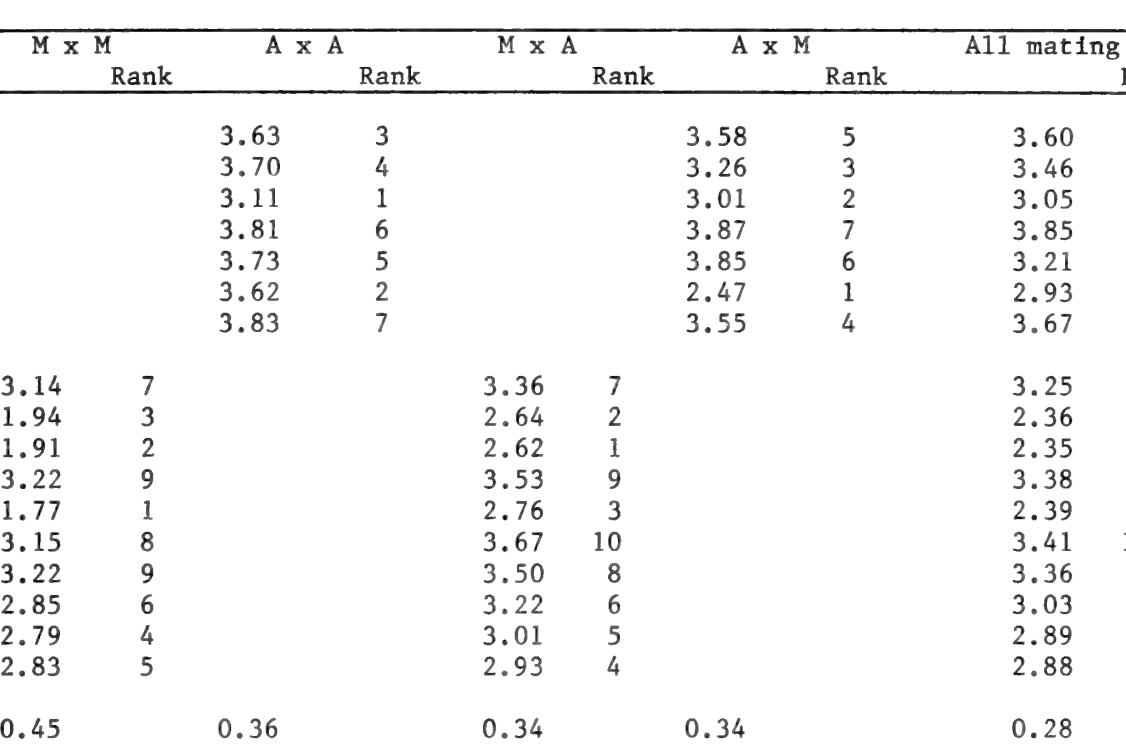

Figure 1.--Lophodermium needle cast attack of Scots pine progenies, differentiated by mating groups ($\bar{x}$ = average of the mating groups).

that, for example, Scots pine clone Fö 45 was less infected than the other Alpine clones, and that clone Fö 51 was one of the most seriously infected. That is in good accordance with the mean ranks of the progenies with these clones as female parent (table 4, fig. 2). Fö 45 ranked in places 1 and 2, whereas Fö 51 ranked in places 6 and 7. Also clone Ber of the Alpine mating group, as male parent, had a very positive effect on the resistance of the progenies.

Within the Mazurian clonal parents the mean scores of the progenies of Re 2, Re 3, or Re 7 were lower than those of the other clones (table 4, fig. 2).

General and Specific Combining Ability Effects

Obviously, the parents had a direct and significant influence on the reaction of a given progeny to the Lophodermium attack. Therefore, a strong genetic effect for the needle cast resistance can be assumed. The analysis of variance of the parent clones in the mating groups provided a test for general combining ability (GCA) and specific combining ability (SCA) (table 5). The results show a very consistent pattern of highly significant GCA effects in all mating groups. The SCA effects were weak or not significant, and highly significant only in Mazurian x Alpine mating group. Thus, in each mating group there were some parent clones that were more resistant or more susceptible than the average (see also fig. 2). The significant SCA effect in the Mazurian x Alpine mating group was caused, presumably, by the very positive influence of the Alpine male clone Ber (table 4, fig. 2). However, the analyses showed that an additive mode of gene action for the Lophodermium needle cast resistance is evident. The family means are very well represented by the sum of their parent effects.

There was also a fairly good heritability of disease resistance with values of h^2n.s. from 0.32 to 0.57 for the four mating groups (table 5).

Table 5.--Results of the ANOVA of the different intraprovenance and interprovenance crossings, based on plot means.

Source	Mazurian x Mazurian			Alpine x Alpine			Mazurian x Alpine			Alpine x Mazurian		
	df	MQ	F-value	df	MQ	F-value	df	MQ	F-value	df	MQ	F-value
GCA	11	44.22	16.44***	8	12.70	9.20***	11	44.33	25.33***	8	32.49	14.70***
female (f)	9	47.08	17.50***	6	8.37	6.07***	9	30.29	17.31***	6	41.02	18.56***
male (m)	2	31.35	11.65***	2	25.70	18.62***	2	107.50	61.43***	2	6.90	3.12*
SCA f x m	15	3.39	1.26 n.s.	9	2.87	2.08*	18	7.99	4.57***	12	1.83	0.83 n.s.
between plots (p)	165	2.69	---	106	1.38	---	185	1.75	---	123	2.21	---
$\hat{\sigma}^2_f$		0.1225			0.0400			0.1274			0.1823	
$\hat{\sigma}^2_m$		0.0435			0.1735			0.2550			0.0145	
$\hat{\sigma}^2_{fm}$		0.0063			0.0335			- 0.0187			- 0.0253	
$\hat{\sigma}^2_P$		0.3622			0.2807			0.2856			0.3248	
$\hat{\sigma}^2_w$ 1)		0.49			0.32			0.58			0.48	
$V_A + V_{AE} = 2(\hat{\sigma}^2_f + \hat{\sigma}^2_m)$		0.3320			0.4270			0.7648			0.3936	
$V_D + V_{DE} = 4\,\hat{\sigma}^2_{fm}$		0.0252			0.1340			0.0000			0.0000	
$V_p = \Sigma\hat{\sigma}^2_i$ 2)		1.0245			0.8810			1.3448			1.0016	
$h^2_{n.s.} \leq (V_A + V_{AE})/V_p$		0.32			0.49			0.57			0.39	

Significance: * ≙ $p \leq 5\%$, *** ≙ $p \leq 0.1\%$, n.s. ≙ $p > 5\%$.
1) The within-plots variance was estimated separately on the basis of the single tree scores.
2) The negative variance components are replaced by zero.

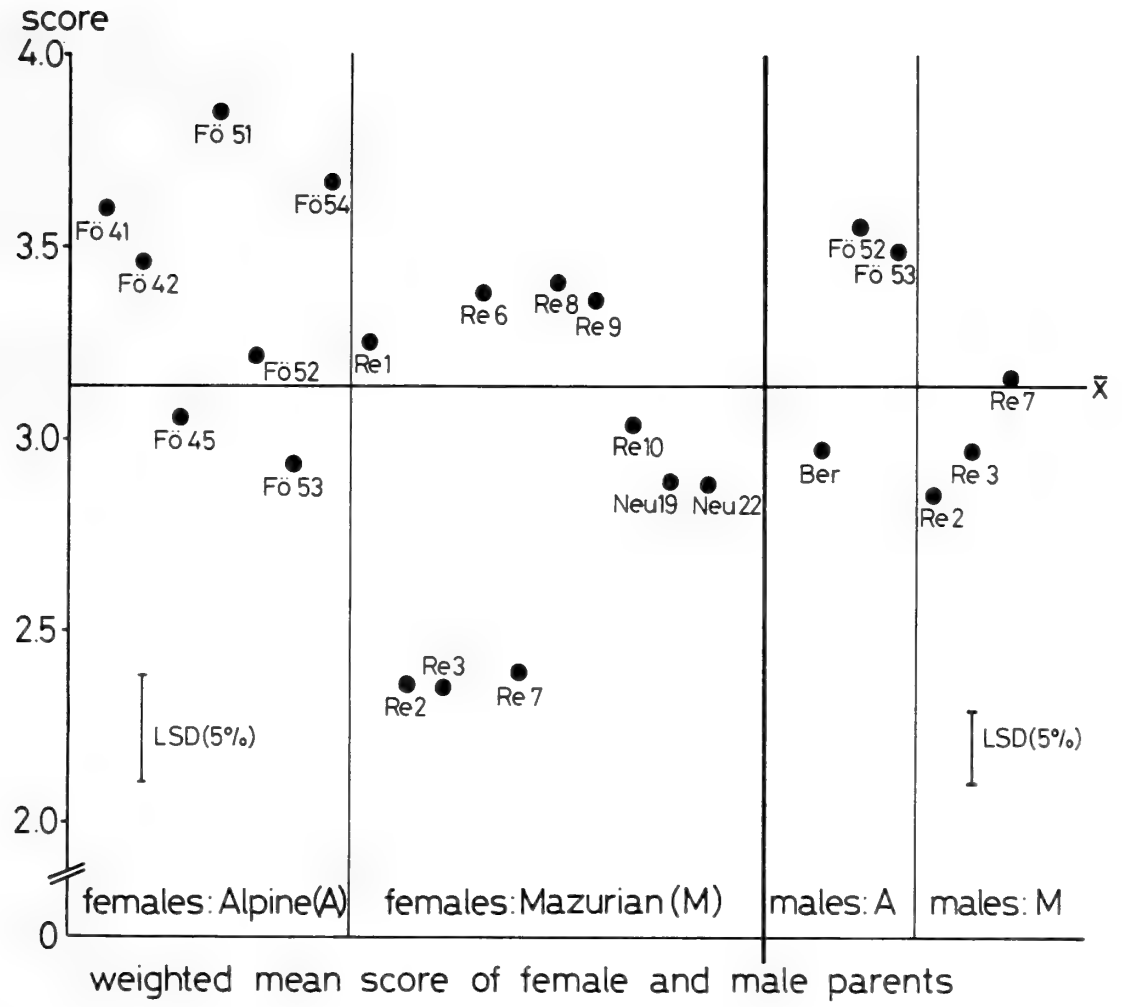

Figure 2.--Weighted mean scores of Lophodermium needle cast attack of female and male parents, differentiated by mating groups ($\bar{x}$ = average of the trial).

DISCUSSION

The results of this study confirmed that northern and northeastern Scots pine provenances (here those from the Mazurian region) possess a higher resistance to Lophodermium seditiosum than southern provenances (from the Alps), when grown in central Europe (Schütt 1958/59, 1964). A significant variation of the needle cast resistance was also found between single trees (parents) and between families, even within the same provenance. Some crossings in a generally more susceptible provenance showed even a higher resistance than susceptible crossings in a resistant provenance (fig. 1).

The great influence of environmental factors could also be confirmed, although the reactions of the progenies on the two tested locations corresponded very well and interaction effects were in general low. Nevertheless, the results should not be generalized. They are valid only for the region of the test. Differences between sites can have many causes. For instance, the conditions for the needle cast disease were obviously more favorable in the field trial Syke. It might also be possible that another composition of the fungus flora existed there. In addition to Lophodermium seditiosum and other Lophodermium species, very often another needle disease pathogen, Naemacyclus minor Butin, was isolated from infected needles in the trial Syke. The reaction to this pathogen must not necessarily correspond with the resistance to Lophodermium. Though, Wilcox (1982) suggested a positive genetic correlation between susceptibility to Dothistroma pini Hulbary and Naemacyclus minor in Pinus radiata D. Don. That may be true also for Lophodermium and Naemacyclus in Pinus sylvestris. Finally, pathogenic variation of Lophodermium seditiosum might have caused differences and interactions between the locations (Hattemer 1965). Nothing is known about races in Lophodermium although cultural variability is evident (Stephan 1975b).

In these studies the resistance of Scots pine to the Lophodermium needle cast disease behaved as a quantitative or polygenic trait, as had been suggested by Langner (1952), Hattemer (1965), Johnsson (1976), and other authors. Continuous variation of the trait resistance even within plots, and a low or lacking dominance variance indicate polygenic inheritance and additive genetic effects (Falconer 1960). One can assume that resistance is controlled by large numbers of genes, which have additive effects. Therefore, resistance of Scots pine to Lophodermium seditiosum can be called "horizontal" in the sense of Robinson (1976). This kind of resistance has a higher security against racial variation in the pathogenicity of the parasite.

The strong additive genetic variance shown by the significant values of the general combining ability, together with the relatively high heritability, indicate that the selection of needle cast resistant trees and their random mating in a seed orchard would give improved resistance in the offspring. Resistant parents can be expected to yield a high frequency of resistant offsprings. This result is in accordance with observations of Johnsson (1976) and Martinsson (1979).

It should be mentioned that the genetic interpretation of the intraprovenance variance components rests on the assumption that the parent trees are a random sample of a population. This assumption seems justified in our case, because the parent trees were not selected for disease resistance. Resistance must be seen in connection with the breeding for other economically important characteristics, e.g., height and diameter growth, form traits, etc. In our crossings the higher needle cast resistance of the Mazurian progenies corresponds very well with their better juvenile height growth (Stephan and Scholz 1980). Therefore, needle cast resistance can be incorporated successfully into further breeding programs with Scots pine.

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Cyclaneusma Needlecast in Pennsylvania: A Review[1] [2]

N. G. Wenner and W. Merrill[3]

Abstract.--The Cyclaneusma needlecast that occurs on Pinus sylvestris in Pennsylvania combines the multiple infection periods and variable, extended incubation periods of the pathogen with the multiple, perennial complements of continuously susceptible host needles. Several distinct but interacting epidemics may occur simultaneously in a plantation, creating a complex epidemiological pattern.

Cyclaneusma minus (Butin) DiCosmo, Peredo & Minter (=Naemacyclus minor Butin) [Ascomycetes: Rhytismataceae)] attacks the needles of various Pinus spp. throughout the world, causing discoloration and premature casting. Taxonomy and nomenclature of this pathogen are reviewed by Dicosmo et al. (1983), host range and geographic distribution by Butin (1973), Miller and Minter (1980), and Osorio and Rack (1980).

ECONOMIC IMPACT

Commercial Christmas tree growers in Pennsylvania harvest approximately 3 million trees per year, valued at $45 million wholesale, and maintain about 12 years of growing stock. Most plantations utilize abandoned farmlands or strip mine spoil banks where soils are unsuitable for conventional agriculture. Pinus sylvestris L. is an excellent species for such poor sites, and in the early 1970's comprised about 75% of the total acreage in commercial production.

In 1972 an unknown needlecast was noted affecting P. sylvestris Christmas tree plantations in Pennsylvania. The disease apparently had been present for several years but had been confused with needlecast caused by Lophodermium pinastri (Schrad. ex Hook.) Chev., with damage caused by the two-spotted pine aphid, Eulachnus agilis (Kaltenbach), or attributed to "premature needle senescence" (Kistler and Merrill 1978b). This disease was first attributed to Naemacyclus niveus (Pers. ex Fr.) Fuck. ex Sacc. (Merrill and Kistler 1974) [=C. niveum (Pers. ex Fr.) Dicosmo, Peredo & Minter], and after Butin's work appeared, to N. minor (Kistler and Merrill 1977a). By 1974 the disease was present throughout Pennsylvania, with >60% of the trees affected in most plantations. Now diseased needles can be found on virtually any P. sylvestris in the state, from seedlings to mature trees on good or poor sites.

Cyclaneusma needlecast is especially destructive to P. sylvestris Christmas trees because it can attack needles of any age on trees of any size over a wide range of environmental conditions. Trees with severe Cyclaneusma needlecast lose all but first-year needles and have reduced quality due to a thin and "hollow cone" appearance. In 1977 one Pennsylvania grower estimated losses due to Cyclaneusma needlecast were about $1,135 per hectare, or about 12% of the gross wholesale value. The disease has intensified and rapidly driven P. sylvestris out of commercial production in most of the state; P. sylvestris accounted for only about 22% of total production in 1982 and growers are planting less acreage of this species each year.

ISOLATION TECHNIQUES

The development of reliable and replicable isolation techniques were fundamental. A detailed description of these techniques is given here, as they form the basis upon which most of our research rests.

Sample trees at least 1 m tall are located at random and permanently tagged in plantations known to have Cyclaneusma needlecast. Plantations with other foliage diseases are avoided to prevent pathogen interactions. Beginning with needle emergence from the fascicle sheath, and at 2- to 3-week intervals thereafter, four twigs are removed at 0.5 meters, one from each cardinal direction on each tree. If these twigs are not to be processed immediately, they are sealed in

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plastic bags and stored at -20°C. We have noted no change in percentages of recovery of C. minus from needles stored frozen for 2 weeks.

Ten needles are randomly removed from the complement of interest on each twig, surface sterilized for 90 seconds in 0.52% aqueous sodium hypochlorite, and rinsed three times in distilled water. Each needle then is cut into three pieces and the three pieces are pressed slightly into the surface of 2% acidified malt agar (AMA = 20 g Difco malt extract, 15 g Difco flake agar, 1 liter double-distilled water, 1.0 ml of 88.3% lactic acid added after autoclaving). The cultures are incubated for 21 days at 21°C in diffuse light. Results are recorded as the number of needles infected out of 40 per tree. In nearly all studies we have used at least 10 sample trees per treatment. Thus, our data normally are based on 400 needles per sampling time per treatment. We have isolated periodically from specific needle complements on individual trees from the time the needles emerged until they were cast, in some cases more than 24 months later. To date we have isolated from approximately 750,000 needles. The large and frequent samplings have been made to counteract tree-to-tree variations in susceptibility.

MORPHOLOGY ON VARIOUS HOSTS

In collections of diseased P. sylvestris needles from all areas of Pennsylvania, the apothecia, asci, and ascospores have had the following average dimensions: 226 X 493 µm, 10.5 X 108.5 µm, and 3.2 X 87 µm, respectively. Pycnidia have not been observed on needles, but form in culture, producing pycnidiospores averaging 1.1 X 7.7 µm. Cyclaneusma minus also has been found on P. nigra Arnold (Merrill et al. 1979), P. ponderosa Laws., and P. virginiana Mill. in Pennsylvania. Length of pycnidiospores produced in cultures obtained from these three hosts averaged 7.5 (4.0-12.8), 6.3 (3.9-10.8), and 6.2 (3.9-10.8) µm, respectively. The sizes of the apothecia, asci, and ascospores on all three hosts fell within the reported range for C. minus. Peterson (1981) also reported C. minus from P. ponderosa in Nebraska, but gave no dimensions of pycnidiospores.

GROWTH IN VITRO

Cardinal temperatures for in vitro growth on AMA under diffuse light for 7, 14, and 21 days were <2:25:>35°C, and <2:21:>30° for in vitro development of apothecia. The cardinal temperatures for in vitro release of ascospores from infected needles and germination of ascospores on AMA were <2:22:30°C (Kistler and Merrill 1977b).

KOCH'S POSTULATES

Proof of pathogenicity was accomplished on 4-year-old greenhouse-grown P. sylvestris (Kistler and Merrill 1977a). Isolations proved the needles were free of previous Cyclaneusma infection. All watering was conducted from the bottom so that the foliage was never wetted, which prevented natural infection. Symptomatic needles bearing apothecia were collected in early June from the field and used as a source of inoculum. The needles were soaked with distilled water and placed over the current year's developing foliage of the potted seedlings. The seedlings were misted with water and placed within large plastic bags in a shaded portion of the greenhouse for 24 hours. The bags were removed and the seedlings were maintained in the greenhouse for 18 additional months. Symptoms first appeared 14 months and apothecia 15 months after inoculation. Reisolations from symptomatic 16-month-old needles yielded C. minus. Needles from similarly treated, but non-inoculated trees, yielded only an occasional isolate of a fungus tentatively identified as Hormonema dematioides Lagerb. & Melin.

SYMPTOM DEVELOPMENT IN THE FIELD

There often are three distinct "flushes" of symptom development on any single needle complement on P. sylvestris in Pennsylvania (Zang 1984). Small numbers of needles may develop symptoms in June-mid-July and in late July-August of the second growing season. The largest number of needles develop symptoms in September-November of the second growing season. However, some needles may not develop symptoms until spring, summer, or fall of the third growing season. We believe that these flushes of symptom development are due to differences in time of infection.

Symptoms have never been observed on field-grown P. sylvestris in Pennsylvania until summer of the second growing season, that is, at least 13 months after needle emergence, even though these needles become infected during the first growing season. We cannot explain the variance between our observations and those of some European workers who report symptoms in 2-3 months (Karadzic 1981; Karadzic and Zoric 1981). The first symptoms appear as small, light-green spots which gradually lighten and coalesce, turning the entire needle a dusty-yellow with distinct transverse brown bars. Such bars occur only rarely on P. nigra, and are indistinct on P. ponderosa and P. virginiana. The first needles to show symptoms usually are those at or just below a node. Eventually symptoms appear on part or all of the remaining needles on the internode. These needles gradually turn a tannish-brown. Needles becoming symptomatic during the summer months usually are cast during the summer or fall. Those needles becoming symptomatic during the fall may be cast during the fall or winter, or may remain attached to the tree through the following spring.

APOTHECIAL DEVELOPMENT AND MATURATION

Apothecia usually begin to appear about 1 month after symptom development. Needles becoming symptomatic in June-early July bear apothecia in late June-early August; needles becoming symptomatic in August-September bear apothecia in September-October. Such apothecia readily release their spores, most of which are germinable on media. Needles becoming symptomatic from early September through October develop apothecia which may release some ascospores in late November-early December. Squash mounts of such apothecia often reveal asci and ascospores which appear mature, but most such asci do not eject their spores and many of those spores which are released do not germinate. Scanning electron microscopy of such apothecia showed that the visible hymenia consisted primarily of paraphyses (Braen and Merrill 1981). These apothecia continued to mature in late winter-early spring when temperatures were still low, but rising. Asci began to protrude above the tips of the paraphyses in March. These asci continued to expand, and the tips became swollen. A few asci liberated spores during rains in late March-early April. Most asci liberated their spores during rains in late April-May. Isolations showed a sharp increase in needle infection occurred immediately after this episode of spore liberation.

Needles becoming symptomatic in the fall may remain attached to the tree, fall to the ground, or lodge within the tree. Apothecia continue to form on these needles throughout the winter and following spring. Growth chamber studies by Zang (1984) indicated apothecial development is affected primarily by a non-linear interaction of temperature and time. Needles maintained at 2°C for 7 weeks formed as many apothecia as needles maintained at 16°C for 2 weeks. The threshold temperature for apothecial development was calculated to be -4°C. Although moisture was necessary for apothecial development, once the needle moisture content was above a threshold level (approximately 50% of the oven-dry weight of the needles) the number of apothecia formed per gram of oven-dry needles was not affected by fluctuations in moisture content over a 2-19°C temperature range.

Winter development and maturation rates of apothecia of C. minus are important as they determine the onset of spore release for the spring infection period. In northern latitudes snow may provide sufficient moisture and insulation from ambient conditions to allow apothecial development throughout the winter. Conversely, in southern latitudes ambient temperatures may remain sufficiently high that apothecia can develop and spores can be released throughout the winter months. Thus the timing of the infection periods in other latitudes or climatic zones may vary considerably from that in Pennsylvania, as indicated by the data of Rack (1981).

SPORULATION

Cyclaneusma minus can release ascospores nearly year round. In the laboratory, viable ascospores were released from apothecia on needles from 2° to 30°C (Kistler and Merrill 1977b). Zang (1984) trapped ascospores in an infected P. sylvestris Christmas tree plantation at temperatures from 2° to 31°C from 1 April to mid-December 1980. Rainfall was the most critical factor affecting spore release. Generally, spore release peaked 2-4 hr after the onset of rain. However, after heavy rains associated with severe thunderstorms at high temperatures, spore release peaked 1 hr after the onset of rain. Spores impacted singly during and following light rains, but in groups of up to eight after sudden, heavy rains. These results were similar to those of Pawsey (1967) who worked with (?) C. niveum. The least amount of rainfall associated with spore release was 1 mm. However, because the collection efficiency of the Rotorod traps used by Zang was only about 30% for C. minus ascospores, spore release may occur with less rainfall (Zang 1984). Indeed, trace amounts of rain triggered spore release three times, but only when the needles were still wet from rainfall the previous day. [Pawsey (1967), perhaps using more efficient spore traps, reported spore release in (?) C. niveum with rainfall considerably less than 1 mm]. Primary and secondary spore release peaks occurred during prolonged rains, indicating successive spore maturation and liberation. The amount of rainfall was unrelated to the number of spores collected. Of spore catches greater than 30 spores/m^3/2 hr, 40% occurred at temperatures from 10° to 16°C. Although more spore release episodes occurred from 0400-0800 and 1800-2000 hr, this appeared due to the daily pattern of rainfall, rather than to a photoperiodic effect. Large spore catches were made in darkness when rainfall also began after darkness.

Considering the season-long trends in spore release, 56.5% of the spores were collected from 3 April to 20 May (fig. 1). Spore release declined through June and became sporadic in late July-early August, sometimes not occurring despite

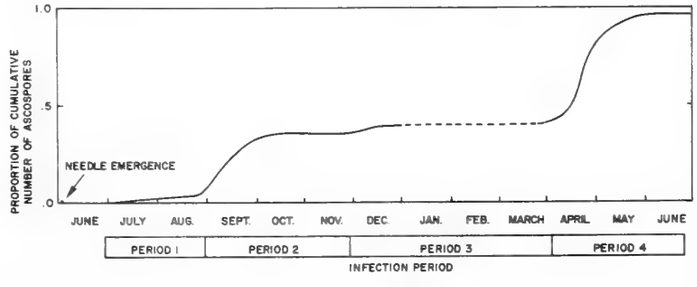

Figure 1.--Hypothetical Cyclaneusma minus inoculum availability curve for one year's complement of Pinus sylvestris needles in Pennsylvania. Dashed line indicates no spore trapping data exists from mid-December through March.

sufficient rainfall. This indicated that the source of inoculum was being depleted; field observations confirmed this. Spore release increased slightly during late August (7% of the total spores), indicating a new source of inoculum. From 2 September through 8 November, 35% of the total spores were collected. Spore release became sporadic again in late October-early November, indicating that this latter inoculum source was being depleted. Another source of inoculum became available from 8 November through 15 December, accounting for 1.5% of the total spore catch. Onset of cold temperature prevented further spore release from this source until the following spring, when the overwintering apothecia of the last inoculum source in 1980 became the first inoculum source in 1981.

INFECTION

Pinus sylvestris needles become susceptible to infection in July (fig. 2) of the first growing season and remain susceptible until they are naturally cast. In Pennsylvania, four distinct infection periods, separated by source of inoculum involved as well as by time of infection, may occur within any 12-month period (Merrill 1982; Merrill et al. 1980b). The first period occurs from mid-July to August and usually accounts for about 5% of total infection. A second infection period occurs from September through November and may account for 0-60% (usually about 5%) of total infection. The third infection period may occur in late November-early December and usually is insignificant. These three infection periods during the first growing season usually account for 10-35% of the total infection, but some years may account for >60% of the total infection. The fourth infection period usually begins about mid-April and extends through June of the second growing season. This period normally accounts for >50% of total infection. Climatological requirements for infection are unknown but moisture probably is the most critical factor. Summer and early fall infection periods are eliminated by rain-free weather, despite the presence of abundant inoculum.

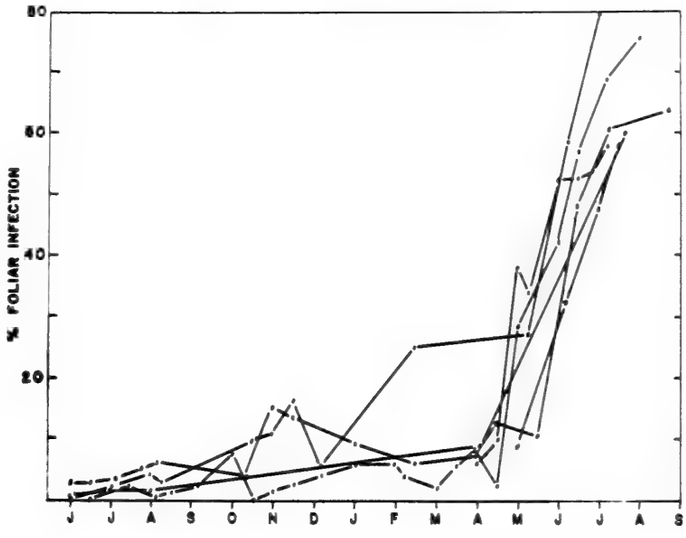

Figure 2.--Six disease progress curves for infection of 1976, 1977, or 1978 needles in six Pinus sylvestris plantations in three areas of Pennsylvania. Each point is the average percentage of infection based on isolations from forty needles from each of ten trees.

INCUBATION PERIOD

The incubation and latent periods of C. minus remain the most confused aspects of the disease cycle. Karadzic (1981) and Karadzic and Zoric (1981) inoculated 2-yr-old potted P. sylvestris seedlings with suspensions of ascospores produced in pure culture and reported symptom development in 2-3 months and apothecial development in 4 months. In contrast, in our studies, potted greenhouse-grown 4-yr-old P. sylvestris seedlings, inoculated with apothecium-bearing needles, developed symptoms in 14 months and apothecia in 15 months (Kistler and Merrill 1978b). In nature we have never seen symptoms on needles less than 13-14 months old, even when isolations showed that significant percentages of such needles were infected shortly after needle emergence. Rack (1981) reported that in Germany apothecia rarely develop on needles less than 15 months old. On the other hand, in Pennsylvania 10- and 11-month-old P. sylvestris needles infected in April-May of the second growing season develop symptoms 5-6 months later in September-October of the second growing season. Workers in New Zealand reported that genetic variability plays a role, and that nitrogen levels affect the rate of symptom development (Gadgil 1977). We applied up to 4X recommended rates of both ammonium and urea nitrogen to infected 1.5-m-tall P. sylvestris with no effect on symptom development (Zang and Merrill, unpublished). Also, we found no differences in time of symptom development in 12 provenances of P. sylvestris (Merrill and Slover unpublished). Evidence thus far suggests that the length of the incubation period in nature varies, due to the age of the needles at the time of infection, the time of year that infection occurs, the amount of environmental stress to which the infected tree has been subjected, or possibly an interaction of these factors.

CONTROL

In field trials, mancozeb (Merrill et al. 1980a), difolatan (Zang and Merrill 1980) and chlorothalonil (Merrill et al. 1982) prevented infection. A flowable formulation of chlorothalonil provided the best overall control. Most infection can be prevented by seven applications of chlorothalonil with the first application about mid-April and the last about mid-September (Merrill et al. 1984). This spray schedule currently is being tested on a large scale by a commercial grower in central Pennsylvania, employing helicopter application. Economically feasible control may be achieved by fewer applications once the disease cycle is better

understood. This disease has been present in all nursery seedbeds that we have examined. To the best of our knowledge, nurserymen have not yet attempted to control seedbed infection. Thus, the pathogen has been widely disseminated in infected planting stock.

Variations in susceptibility exist both within and among P. sylvestris provenances (Merrill and Slover 1983). Within a plantation of trees planted at the same time from the same seedling lot, it is possible to find trees holding three or four complements of needles adjacent to trees holding only a single needle complement. In a replicated planting of twelve different seed sources, southern European provenances were more susceptible than provenances of unknown origin selected over the years by Pennsylvania nurserymen. Studies are in progress to select resistant individuals within various provenances, with suitable aesthetic qualities, to produce seed trees for "superior" Christmas trees.

DISCUSSION

One is at a loss to interpret some published data, as authors have failed to identify the specific complements of needles being studied, or the level of infection found in other complements of needles on the trees in question. Based on our experiences with this fungus over a 12-year period, pathogenicity trials should be conducted using seedlings that have been maintained under conditions precluding any infection for at least 2 years prior to and after the initiation of the trials. Otherwise, one has no proof of the source of inoculum, or time of infection. High levels of infection may occur in symptomless needles (Kistler and Merrill 1978a). Thus, conclusions based on evaluation of visible symptoms are of dubious value.

The length of the incubation and latent period in nature remain major unanswered questions. Any attempt to explain the disease cycle until these factors are elucidated is mere speculation. The time-temperature-moisture requirements necessary for infection must be determined before the relative importance of control programs directed against the summer-fall infection periods can be assessed, or before the probable importance of the various infection periods in other climatic zones can be estimated.

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Ecology and Succession of Some Fungi Causing Pine Needle Diseases in Yugoslavia[1]

Vladimir Lazarev[2]

Abstract.--The ecological characters and succession of fungi that frequently colonize needles of several pines in Yugoslavia are described. Information on succession of fungi on needles of different ages is presented. Lophodermium seditiosum is considered the most important primary pathogen of pine needles in nurseries in Bosnia and Hercegovina.

INTRODUCTION

Production of pine seedlings in nurseries in Bosnia and Hercegovina (Yugoslavia) has increased in the last decade. Except for spruce, pine is the most important species grown. This increase in pine production, development of large nurseries, and new plantations brought problems with diseases of economic importance, including pine needle diseases, the subject of much of our research.

This paper presents the results of recent work on the ecological character and succession of some fungi that frequently colonize pine needles.

MATERIALS AND METHODS

Infected primary and secondary needles of Pinus sylvestris, P. nigra var. austriaca, P. strobus, P. halepensis, P. banksiana, and P. contorta were collected in 13 localities of Bosnia and Hercegovina, from pines in nurseries, plantations, and natural stands.

Collected needles were up to 4 years in age. The following symptoms were analyzed: color changes, fruiting bodies and their appearance, and appearance of cross lines. Pure cultures obtained from green and necrotic needles were also analyzed; and, according to results of the isolations, conclusions about succession were made.

Both primary and secondary needles were analyzed. These needles were divided into six groups, according to their age and health:

1. Old primary needles from second-year seedlings. These needles change their color after 2 months (becoming brown).
2. Young primary needles from second-year seedlings. These needles change color soon after appearance of secondary needles.
3. Secondary needles from current vegetation primarily infected by Lophodermium seditiosum Minter, Staley & Millar or Lophodermella sulcigena (Rostr.) Höhn.
4. Secondary needles, third-year and older, in which physiological processes are gradually reduced. These needles change color during summer; they become yellow and are cast in autumn.
5. Secondary needles still attached on damaged plants. Plants damaged by many agents (wind, fire, insects, decay fungi) change their physiological activities, and their needles are usually colonized by some secondary pathogens. These needles might be any age.
6. Secondary early cast needles. Early needle cast could be caused by various agents. Needles fall while still green and then become brown. They also could be any age.

RESULTS

The following fungi were isolated from needles: Lophodermium seditiosum, L. pinastri (Schrad. ex Hook) Chev., L. conigenum (Brunaud) Hilitz., Lophodermella sulcigena, Naemacyclus minor Butin, N. niveus (Pers. ex Fr.) Fuck. ex Sacc., Dothistroma pini Hulbary, and Meloderma desmazierii (Duby) Darker.

The results are presented in table 1. The position of fungi in succession is indicated.

According to our observations, pine needles that belong to ecological groups 1 to 6 were most frequently colonized by Lophodermium spp. L. pinastri usually produced fruiting bodies on old

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Table 1.--Ecology and succession of some fungi causing pine needle diseases.

Locality	Pine species	Ecological groups	Pathogen's name and place in succession[1]
		Nurseries	
Banja Luka	P. sylvestris, P. nigra	2	L. seditiosum x
Banja Luka	P. sylvestris, P. nigra	1	L. pinastri x
Banja Luka	P. sylvestris	3	L. seditiosum x
Doboj	P. sylvestris, P. nigra	2	L. seditiosum x
Doboj	P. sylvestris	3	L. seditiosum x
Doboj	P. sylvestris	4	L. pinastri x
Sokolac	P. sylvestris	2	L. seditiosum x
Sokolac	P. nigra	1	L. pinastri x
Sokolac	P. sylvestris, P. nigra	3	L. seditiosum x
Sokolac	P. sylvestris	4	L. pinastri x
Sokolac	P. sylvestris	4	L. pinastri x, N. minor xx
Olovo	P. nigra	1	L. pinastri x
Olovo	P. sylvestris	2	L. seditiosum x
Olovo	P. nigra	3	L. seditiosum x
Nevesinje	P. sylvestris	2,3	L. seditiosum x
Nevesinje	P. sylvestris	4	L. pinastri x
		Plantations	
Zavidovići	P. nigra	3	L. seditiosum x
Zavidovići	P. nigra	4	D. pini x
Zavidovići	P. sylvestris	5	L. pinastri x
Doboj	P. nigra	5	L. seditiosum x, L. pinastri xx, N. minor xxx
Doboj	P. sylvestris, P. nigra	3	L. seditiosum x
Doboj	P. strobus	5	L. pinastri x
Doboj	P. strobus	4,5	M. desmazierii x
Doboj	P. sylvestris	4,5	L. pinastri x
Doboj	P. sylvestris	4	L. pinastri x, N. minor xx
Doboj	P. sylvestris	5	L. minor x
Doboj	P. nigra	3	L. seditiosum x, N. niveus xx
Maglaj	P. sylvestris	3	L. seditiosum x
Sokolac	P. sylvestris	3	L. sulcigena x
Sokolac	P. sylvestris	4	L. pinastri x, N. minor xx
Sokolac	P. sylvestris	4	N. minor x
Sokolac	P. sylvestris	3	L. seditiosum x, L. pinastri xx N. minor xxx
Sokolac	P. sylvestris	3	L. sulcigena x, L. pinastri xx
Sokolac	P. sylvestris	5	N. minor x, L. pinastri xx
Celinac	P. nigra	4	D. pini x, L. pinastri xx
Celinac	P. strobus	4	M. desmazierii x
Celinac	P. banksiana	4	L. pinastri x
Celinac	P. strobus, P. contorta	5,6	L. pinastri x
Celinac	P. nigra	5,6	L. pinastri x, N. niveus xx
Fojnica	P. sylvestris	3	L. sulcigena x
Box. Novi	P. nigra	4	D. pini x, L. pinastri xx

(continued on next page)

Table 1.--Continued

Locality	Pine species	Ecological groups	Pathogen's name and place in succession[1]
		Natural Stands	
Trebinje	P. halepensis	5	Lophodermium conigenum x
Sarajevo	P. sylvestris	4	L. pinastri x, N. minor xx
Sarajevo	P. sylvestris	1	L. pinastri x
Sarajevo	P. sylvestris	4	N. minor x, L. pinastri xx
Sarajevo	P. nigra	4	L. pinastri x, N. niveus xx
Sokolac	P. sylvestris	1	L. pinastri x
Zavidovići	P. nigra	1,4	L. pinastri x
Bugojno	P. sylvestris	4	L. pinastri x

[1]x = primary pathogen, xx = secondary pathogen, xxx = tertiary pathogen.

primary needles in nurseries and in natural pine stands, on third-year and older secondary needles, and on secondary needles of plants that were damaged by other agents. L. conigenum was found only on the secondary needles of Pinus halepensis that were damaged by other agents. L. seditiosum was usually isolated from young primary needles and secondary needles of current vegetation, but it was also found on the secondary needles of plants that were damaged by other agents.

L. sulcigena infected only needles 1 year or less in age. D. pini was found mainly on second-year secondary needles. N. niveus and M. desmazierii were not found as the primary pathogens on young needles from current vegetation. They were usually found on the needles that belong to group 4 (needles with reduced physiological activity). N. niveus, which frequently follows Lophodermium spp., is considered the secondary pathogen; N. minor infects older needles as a secondary pathogen, rarely as a primary pathogen (they both colonized needles that belong to groups 3, 4, and 5).

The most frequent occurrence of L. pinastri, D. pini, N. minor, N. niveus, and M. desmazierii was on needles of ecological groups 4 and 5, while the needles that belong to group 6 were usually infected by L. pinastri. L. seditiosum and L. sulcigena, as the primary parasites, infect current needles that belong to groups 2 and 3.

Besides these fungi there are some other pathogens that occur sporadically, such as Diplodia pinea (Desm.) Kickx, Encoelia petrakii Gremmen (needle disease of Austrian pine), Thyriopsis halepensis (Cooke) Theiss. (needle disease of Pinus halepensis), and also some fungi without particular importance like Sclerophoma pithyophila (Corda) v. Höhn., Coniothyrium fuckelii Sacc., Elytroderma sp., and Gibberella sp.

According to the data presented in the table, there was no succession of pathogens on the primary needles. However, on the secondary needles the succession of fungi does exist and is as follows:

- In nurseries on Scots pine needles third-year and older, L. pinastri and N. minor are in the succession.
- In plantations on third-year needles of Scots and Austrian pine, L. seditiosum, L. pinastri, and N. minor are in the succession. On Scots pine succession could also exist between L. pinastri and N. minor.
- In plantations of Scots pine on second-year needles, L. sulcigena is followed by L. pinastri.
- In plantations of Austrian pine, D. pini is followed by L. pinastri on second- or third-year needles.
- In plantations of Austrian pine on third-year needles, L. seditiosum is followed by N. niveus. On these or older needles, L. pinastri could be followed by N. niveus.
- In Scots pine stands on third-year needles, L. pinastri is followed by N. minor; and on these or older needles, N. minor could be in succession with L. pinastri.
- In Austrian pine stands on third-year needles, L. pinastri is followed by N. niveus.

Some needles of Scots pine infected by L. sulcigena are colonized by Hendersonia acicola Tub. as the secondary pathogen, but it does not cause earlier needle cast. Needles infected only by L. sulcigena are cast at the same time as those infected by both L. sulcigena and H. acicola.

CONCLUSIONS

Among fungi colonizing needles of pine seedlings in nurseries in Yugoslavia, the most important primary pathogen is L. seditiosum, which

infects both current young primary needles of second-year seedlings and current secondary needles of older plants. Confusion could arise regarding L. pinastri, which is usually found on the needles of second-year seedlings (primary needles). However, the needles that are infected by L. pinastri are the first ones--oldest (older primary needles)--and they have no important effect on further growth of plants. These needles likely lost their vigor before they were infected by L. pinastri. Therefore, there is no succession of fungi on primary needles.

The secondary needles of young plants in nurseries usually are infected first by L. seditiosum, which is then often followed by L. pinastri and N. minor (in case of Scots pine). L. pinastri could infect needles that have not been first colonized by L. seditiosum but that have weakened physiological activities.

Among fungi that colonize current secondary pine needles in plantations, L. seditiosum and L. sulcigena are found as primary pathogens. L. pinastri, N. minor, D. pini, and M. desmazierii could infect needles that are not primarily colonized by L. seditiosum and L. sulcigena but that have weakened physiological activities. In succession with L. seditiosum, the following fungi are found as secondary pathogens: L. pinastri (Scots and Austrian pines); N. minor (mainly Scots pine); N. niveus and D. pini (Austrian pine). In succession with L. sulcigena the secondary pathogen is usually H. acicola. In case of Pinus strobus, M. desmazierii is found alone on needles with weakened physiological activities. All other fungi that are found in succession are of less important.

The secondary needles in natural stands are colonized mainly by L. pinastri (Scots and Austrian pines), but also by N. minor (Scots pine), N. niveus (Austrian pine), and L. conigenum (Pinus halepensis). It is noted that L. pinastri and Naemacyclus spp. may succeed one another.

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Lophodermella Species on Pines[1]

C. S. Millar[2]

Abstract.--Research work on two European and five North American species of Lophodermella is reviewed. Differences in geographical distribution, host, ascocarp development, ascospore size, production of pycnidia, and time for symptom development and needle cast are noted. Species are similar in the time of sporulation, which is synchronized with new needle growth, and type of germination of ascospores and infection process. Lophodermella species fall naturally into three groups: L. sulcigena, L. morbida, and L. montivaga, with short spores and autumn lesion development; L. arcuata and L. concolor, with short spores and spring lesion development; and L. cerina and L. conjuncta, with long spores and summer lesion development one year after infection. A new species, L. maureri, is introduced.

INTRODUCTION

Lophodermella, a genus erected by von Höhnel in 1917, comprises eight species, all of which are on pines. Darker (1932) recognized six species of Hypodermella v. Tub. on pines and later transferred these to Lophodermella (Darker 1967). Subsequently, L. morbida (Staley and Bynum 1972) and L. maureri (Minter, unpublished) were discovered.

In 1932, Darker thought that the species of Hypodermella were, without exception, worthy of more detailed study because every species appeared to be an active parasite. Among those on pines, all have been studied further; still, relatively little is certain about the way they infect needles, the variation in and between their life cycles, their effect on tree growth and our ability to control them.

This review will highlight some of these gaps in our knowledge and compare the two European and six American species in the hope that this might identify fields for further study.

GENERAL CHARACTERISTICS

Lophodermella species bear their ascocarps singly in a simple stroma which is sub-hypodermal in the needle tissue. The ascospores are clavate with a mucilaginous sheath and often become once septate at maturity. Germination is by a single germ tube, from one or both cells, which soon forms an appressorium from which a peg appears to originate to penetrate the needle cuticle (Millar 1981, Staley 1978). All species infect only needles, on which symptoms and ascocarps develop at varying rates. Some species produce pycnidia. Infected needles are cast soon after ascocarp maturation whether or not the spores are discharged. Several secondary colonizers of lesions of Lophodermella, especially species of Hendersonia Sacc., prevent the primary pathogen from fruiting and thus effect some biological control (Darker 1932).

SPECIFIC CHARACTERISTICS

The distribution and main specific morphological and developmental characters of the Lophodermella species on pines are summarized in tables 1 and 2, in which the species have been arranged in an order that brings apparently similar species together. The main criteria used are spore size and life cycle.

Reviews of the work on individual species are presented separately first and then discussed collectively.

Lophodermella sulcigena (Rostr.) Höhn.--This fungus is found throughout most of Europe from Yugoslavia north to Scandinavia on Pinus mugo and P. sylvestris, and on P. nigra var. maritima (Corsican pine) where it has been introduced into the Netherlands and Britain. Records on P. halepensis (Robak 1963) and P. contorta (Kujala 1950) are doubtful, especially on the latter since Karlman (1980) states specifically that P. contorta is not infected in Scandinavia. The record on P. radiata attributed by Gibson (1979) to Scott (1960) is inaccurate.

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Table 1.--Distribution and morphology of *Lophodermella* spp. on *Pinus* spp.

Lophodermella	*Pinus*[1]	Needles	Subsection[2]	Distribution	Ascospores (µm)	Pycnidia	Main reference
L. sulcigena	(*P. halepensis*)	2	Sylvestres	Europe	-	Unknown	Robak 1963
	P. mugo	2	Sylvestres	Europe	40-60	Unknown	Prihoda 1949
	P. nigra	2	Sylvestres	Europe	26-(30-42)-46	Unknown	Millar & Minter 1978
	P. sylvestris	2	Sylvestres	Europe	27-(45-54)-65	Unknown	Terrier 1944
	(*P. contorta*)	2	Contortae	-	-	-	Kujala 1950
	(*P. radiata*)	3	Oocarpae	-	-	-	Gibson 1979
L. morbida	*P. ponderosa*	3	Ponderosae	W. U.S.A.	23-53	Present	Harvey 1976
	P. attenuata	3	Oocarpae	W. U.S.A.	-	-	Staley & Bynum 1972
L. montivaga	*P. contorta*	2	Contortae	W. U.S.A.	32-(39)-50	Unknown	Sydow & Petrak 1922
	P. sylvestris	2	Sylvestres	-	-	-	Hunt & Ziller 1978
	P. ponderosa	3	Ponderosae	W. U.S.A.	-	-	Gibson 1979
	P. radiata	3	Oocarpae	W. U.S.A.	-	-	Offord 1964
	P. monticola	5	Strobi	W. U.S.A.	-	-	Hunt & Ziller 1978
	P. flexilis	5	Strobi	W. U.S.A.	-	-	Hunt & Ziller 1978
L. arcuata	*P. albicaulis*	5	Cembrae	W. U.S.A.	-	Unknown	Hunt & Ziller 1978
	P. monticola	5	Strobi	W. U.S.A.	34-37	-	Thyr & Shaw 1966
	P. lambertiana	5	Strobi	W. U.S.A.	42-50	-	Darker 1932
	P. flexilis	5	Strobi	W. U.S.A.	-	-	Thyr & Shaw 1966
L. concolor	*P. banksiana*	2	Contortae	W. U.S.A.	-	-	Mielke 1956
	P. contorta	2	Contortae	W. U.S.A.	45-60	(Present)	Franc 1977
	P. sylvestris	2	Sylvestre	Canada	-	-	Hunt & Ziller 1978
L. cerina	*P. taeda*	3	Australes	S. U.S.A.	-	-	Czabator et al. 1971
	P. elliotii	3	Australes	S. U.S.A.	-	-	Czabator et al. 1971
	P. ponderosa	3	Ponderosae	W. U.S.A.	-	-	Staley 1979
	P. contorta	2	Contortae	W. U.S.A.	68-78	Present	Darker 1932
	P. sylvestris	2	Sylvestres	-	-	-	Staley 1979
L. conjuncta	*P. mugo*	2	Sylvestres	Europe	-	Unknown	Terrier 1944
	P. nigra	2	Sylvestres	Europe	-	Unknown	Mitchell et al. 1978
	P. sylvestris	2	Sylvestres	Europe	45-(75-90)-100	Unknown	Wilson 1922
L. maureri	*P. ayacahuite*	5	Strobi	Mexico	33-50	-	Minter, unpublished

[1]Parentheses around *Pinus* spp. indicate incorrect or doubtful records.
[2]Subsections of *Pinus* are from Critchfield & Little (1966).

Table 2.--Symptoms and life cycles of *Lophodermella* spp. on *Pinus* spp.

Lophodermella spp.	Sporulation time	First symptoms	Ascocarp development	Needle cast	Associated fungi
L. sulcigena	June-August	After 1 month	November onwards	After c. 15 months	*Hendersonia acicola* *Hendersonia montana* *Lophodermium* spp.
L. morbida	June-August	After 1 month	November onwards	After 13-15 months	*Hendersonia* sp.
L. montivaga	July-September	After 1 month	Over winter	-	-
L. arcuata	July-August	After 9 months	After 11 months	After 16 months	-
L. concolor	June-July	After 9 months	After 11 months	After 12-14 months	*Hendersonia pinicola* *Hemiphacidium longisporum*
L. cerina	-	Autumn	Spring	After 1-2 years	*Hendersonia acicola* *Lophodermium australe* *Ploioderma lethale* *Ploioderma hedgcockii*
L. conjuncta	All year with peak June-Sept.	After 12-18 months	After 12-24 months	After 2 years	*Hendersonia acicola* *Phaeoseptoria* sp.
L. maureri	-	-	-	-	-

The conspicuous brown- to violet-coloured hysterothecia develop from November over winter on first-year needles; these are browned usually to within 5-10 mm of their bases, which may remain green. Ascospores mature in March-June in Italy (Moriondo 1963), but in June-August in Scotland, and infect new needles at their existing bases (Millar 1970). Symptoms are first seen about one month later and lesion extension is usually complete by October. Descriptions have been given from Rostrup (1883) onwards (Jalkanen 1981). Needles are cast in October in Britain but may be cast earlier in more continental climates (Hanso 1970).

Infected trees appear pinkish brown and are usually scattered in any stand. There is some suggestion that edge trees are affected more than trees inside a stand (Lagerberg 1910, Terrier 1944, Robak 1963) but the evidence is doubtful. Trees of all sizes are infected, but those 10-30 years old in dense plantations seem to be more susceptible (Jørstad 1925). Terrier found that whole stands of Scots pine, both in plantations and nurseries in Switzerland, could succumb to the disease; but, I found L. sulcigena on Scots pine only twice in a single nursery and only scattered in plantations in Scotland. Mitchell et al. (1976a) showed that the volume of diseased Corsican pine trees was reduced by 59% because of a 6-year-long epidemic, but height growth was not affected. However, Lagerberg (1910) noted a considerable decrease in the growth of shoots of infected trees, and Terrier (1944) reported a severe decline in length and thickness of the trunk and branches.

Few details of experimental work with L. sulcigena have been published. However, it is known that spores are ejected singly and forcibly from the ascus about 2 mm in humid conditions, and germinate only at the base of flushing needles to form a short germ tube and an appressorium (Campbell 1972). Infection is through the cuticle by means of an infection peg from the appressorium (Millar 1970). In vitro, ascospores germinate over the range 3-30° C (optimum 23° C). The germ tubes lyse at 30° C.

Gordon (1966) classified the ascocarp development of L. sulcigena on an unspecified host as Type 1 with pseudoparaphyses; but Campbell and Syrop (1975), working with Corsican pine needles, detected true paraphyses and could not, therefore, fit it into Gordon's classification. Campbell (1973) studied the ultrastructure of asci, ascospores, and spore release and found that asci had a simple apical ring through which spores were released one at a time. Minter and Cannon (1984) have confirmed this.

There is clear evidence of resistance to L. sulcigena both within and between provenances of Scots pine. Lagerberg (1910) found that northern provenances were more resistant than southern ones when the two were grown in the north of Sweden; Jalkanen (1982) has confirmed this with Finnish provenances, but the relation is not linear or absolute as some resistant provenances occur in the south. Many authors have noted considerable variation within a stand. Jalkanen et al. (1981) found no correlation between wax formation on needles of different provenances and resistance to L. sulcigena. Pines planted on fertile sites appear to be more susceptible to L. sulcigena than pines on infertile sites (Krutov 1979), possibly because on fertile sites the level of macro-nutrients in the needles is too high in relation to micro-nutrients (Kurkela and Jalkanen 1981). Lagerberg (1910) suggested that a degree of control might be obtained by removing diseased trees before the maturation of the hysterothecia, and Terrier (1944) recommended burning such material. Chemical control is possible, at least on a single dipped shoot basis (Millar 1970), but Kalandra (1938) did not obtain satisfactory results in a stand, and I have had the same experience when spraying whole trees.

Hendersonia acicola Münch & v. Tubeuf or H. montana Viull. are found regularly associated with needles infected first by L. sulcigena and in some cases fruit inside the hysterothecia of L. sulcigena (Münch and von Tubeuf 1911, Kalandra 1938, Moriondo 1963, Mitchell et al. 1976b).

I have found heavy secondary colonization of L. sulcigena lesions on Corsican pine by Lophodermium seditiosum Minter, Staley & Millar and L. conigenum Hilitzer, both of which sporulate in the autumn when the new lesions of L. sulcigena are just developing.

Lophodermella morbida Staley & Bynum.--L. morbida occurs in Pinus ponderosa plantations in Oregon and Washington west of the Cascades and on P. attenuata in California. This very aggressive parasite (Bynum and Miller 1969) was recorded first in 1953 (Staley and Bynum 1972).

The long hysterothecia, which may be the whole length of the needle, are concolorous to dark brown and fairly conspicuous, especially when dry. They mature from early June to mid-August, corresponding with the period of new needle growth, and infect new needles probably just distal to the fascicular sheath (Staley and Bynum 1972). Symptoms appear soon after infection from July to September as brown lesions that expand to turn the whole needle reddish brown. Ascocarp development starts in October, 4 months after infection, and continues overwinter, so that asci are discernable by April or May of the year following infection. After sporulation of the mature ascocarps, needles are cast. The life cycle is completed in 14-15 months.

Heavily infected trees have a tufted appearance due to the presence of only current year's needles. In some cases trees are killed (Bynum and Miller 1969). Harvey (1976) detected a reduction in height and radial growth in P. ponderosa, but he did not calculate volume changes.

Uecker and Staley (1973) studied the development of ascocarps of L. morbida in P. ponderosa needles and found that it did not fit into any of the categories of Gordon (1966). Harvey (1976), in a detailed study of the biology of L. morbida, found that ascospores were discharged forcibly a few millimetres within 15

minutes of wetting a hysterothecium or within 2-3 hours in a saturated atmosphere. The spores started to germinate within 1 hour but germination ceased if they became dry. Spore discharge was associated in the field with rain and high humidity. Spore trapping and bagging experiments indicated that the infection period preceded the summer drought of western Oregon. Harvey noted that the start of sporulation coincided with the flowering of Rubus parviflorus Nutt. Flexible collodion needle prints (Delp 1954) demonstrated the presence of germination of ascospores on needle surfaces, but the mode of penetration was not determined.

Evidence from Staley and Bynum (1972) and Harvey (1976) suggests that L. morbida is restricted to areas with moist early summer climates, and Harvey noted that clouds which persisted near ridge tops from 760 to 900 m in the western Cascades could account for the belt of disease there.

Staley and Bynum (1972) noted that some individuals within the provenance test planting in which the fungus was first found had survived some 20 years later, indicating a degree of resistance. Harvey (1976) was able to select diseased and healthy trees to make an assessment of the effect of disease on growth, again indicating some resistance within a stand. No work on control has been reported.

Staley and Bynum (1972) found sub-hypodermal pycnidia, probably of a Leptostroma, associated constantly with Lophodermella morbida. The pycnidia formed in September soon after the needles had turned brown. In addition, during and after ascospore discharge, infected needles were colonized rapidly by secondary fungi, particularly a Hendersonia sp.

Lophodermella montivaga Petrak.--This fungus occurs on Pinus contorta in Idaho and Montana (Sydow and Petrak 1922); Wyoming, Oregon, and Colorado (Darker 1932); and Canada (Hepting 1971). Offord (1964) recorded it on P. radiata in California. It is recorded also on P. flexilis, P. monticola, and P. sylvestris (Hunt and Ziller 1978) and P. ponderosa (Gibson 1979), giving it the widest host range of the eight species.

L. montivaga has a 1-year life cycle (Staley and Bynum 1972). The hysterothecia are dark brown to black, 1-4 mm long. No pycnidia have been found. Ascospores mature from July to October and germinate by a short germ tube to produce an appressorium from which an infection peg is assumed to penetrate the cuticle of new needles. The first symptoms appear in September in Colorado; the needles turn brown and ascocarps develop in them over winter (Staley and Bynum 1972, Staley 1978). The symptoms and development and structure of the ascocarp are said to resemble closely those of L. sulcigena (Darker 1932) and L. morbida (Staley and Bynum 1972).

Only occasional infected trees and small centres of infection were observed in Colorado by Staley (1964). The influence on the tree or on stand growth has not been assessed.

Lophodermella arcuata (Darker) Darker.--L. arcuata occurs only on 5-needled pines. It is recorded on P. albicaulis (Hunt and Ziller 1978), P. flexilis in Montana and Colorado (Thyr and Shaw 1966), P. lambertiana in Oregon (Darker 1932) and California (Burleigh et al. 1982), and P. monticola in Idaho where Shaw and Leaphart (1960) described it as a serious foliage disease.

The erumpent, long, concolorous hysterothecia develop rapidly on first-year needles, which turn brown for their entire length in spring. Mature ascospores are released in July and August and infect new needles 2-5 months old. Most needles are cast by September of their second year so that the life cycle is completed in 15-16 months. No pycnidia have been found associated with L. arcuata.

On P. monticola, the fungus affects mainly the middle and upper crowns of pole-size to mature trees (Shaw and Leaphart 1960). In P. flexilis stands, only occasional, isolated trees are affected (Staley 1964). The upper crown of diseased trees is thin, with often only the current year's needles present. On P. lambertiana, most trees in two plantations observed by Burleigh et al. (1982) became infected, and both terminal and radial growth were reduced. The effect of disease on radial growth was, however, confounded by possible drought effects. Disease was relatively unimportant in natural stands.

Little experimental work has been reported for L. arcuata. Thyr and Shaw (1966) studied its development in P. monticola needles and found that ascocarp development starts in April-May about 10 months after infection and conforms to Gordon's Type I (Gordon 1966), in which the filament tips become detached to form the clypeus, leaving the filament bases resembling paraphyses. Young ascospores had a single large nucleus. Mature spores often formed one septum and germinated from either cell by a short germ tube, which enlarged to a variously shaped appressorium. Nuclear division in the ascospore was not described. An apparent penetration peg formed in the side of the appressorium adjacent to the needle, and infection was assumed to be through the cuticle, and small-diameter hyphae were seen in the epidermal cell outer wall.

Staley (1978) also observed appressorium formation on P. flexilis and, although also unable to obtain evidence of penetration, was firmly of the opinion that penetration of the leaf surface took place directly from appressoria.

Although there has been no work specifically on resistance or control, it is clear from the published papers that, even in heavily infected plantations (Burleigh et al. 1982), resistant, healthy trees can be found, whilst in natural stands often only occasional susceptible trees are seen (Staley 1964).

Lophodermella concolor (Dearn.) Darker.--This fungus is widespread and common in North America. It has been recorded from Montana, Idaho, Oregon, Colorado, Wyoming, Utah, and British Columbia on Pinus contorta (Mielke 1956, Ziller and Funk 1973) and from southern Alberta to northern Ontario on P. banksiana (Mielke 1956). Hunt and Ziller (1978) recorded it on P. sylvestris.

It is a strong pathogen with a 1-year life cycle. The small, colourless ascocarps appear as shallow depressions, almost circular when dry. New needles are infected in June and July and turn reddish brown about 9 months later, becoming somewhat greyish when the ascocarps start to mature in May. Infected needles are dwarfed and appear tufted. Infection is more or less even on young crowns, but on older trees the lower crown is most heavily infected, with often only the current year's needles remaining. Needle cast occurs 12-14 months after initial infection at, or slightly before, maturation of the hysterothecia; it leads often to almost complete defoliation, which may result in shoot and branch death (Darker 1932, Mielke 1956). Tree mortality due to L. concolor, if it occurs, will, according to Williams (1976), likely be in highly susceptible, low vigour or stressed trees in overstocked stands and may involve root diseases or bark beetles.

Darker (1932) conducted successful infection experiments on P. banksiana. Ascospores do not germinate freely in distilled water (Darker 1932) but do so on needles, forming a septum, germ tube, and appressorium (Staley 1978). It is not clear from Mielke (1956) whether or not infection experiments have been done on P. contorta.

Severe infection occurs in areas where fog or mist are common (Mielke 1956), and high precipitation probably increases disease (Williams 1976). Resistant trees, which come from among the tallest and most vigorous, have been selected by Franc (1977) for inclusion in a P. contorta improvement programme; but results of any further work on resistance to this disease have not yet been reported.

Possible pycnidia were detected by Darker (1967) in sub-stomatal chambers on an unspecified host but have not yet been confirmed. Hemiphacidium longisporum Ziller and Funk (Ziller and Funk 1973) and Hendersonia pinicola Wehm are secondary fungi which appear to inhibit fruiting of L. concolor. H. longisporum is, however, restricted to a very narrow coastal line not more than one mile from the sea (Ziller and Funk 1973).

Lophodermella cerina (Darker) Darker.--L. cerina is strongly parasitic on Pinus ponderosa in Colorado, Arizona, California, and New Mexico (Staley and Bynum, 1972) and is found also on P. contorta in California (Darker 1932); P. elliotii var. elliotii and P. taeda in the southern states of Louisiana, Mississippi, Alabama, and Florida (Czabator et al. 1971); and P. sylvestris (Staley 1979).

The ascoscarps are scattered, oval, concolorous, and inconspicuous in 10-140 mm bands on 1-year-old needles of P. ponderosa, but on partly or wholly browned needles of the southern pines. Pycnidia are flask shaped or occasionally applanate (Staley and Bynum 1972). There is no information on spore release. Symptoms on first-year needles appear first in November on southern pines, and ascocarps are visible by late February and prominent by the end of March. Czabator et al. (1971) state that two years' needles are affected; however, it is not clear whether this means that there are two sets of infected needles on a tree at the same time. The time of needle cast is not given.

All ages of southern pines are affected but disease is more common in older stands. The infection is scattered in a stand but is all over infected trees. Tree growth studies and research on the etiology of L. cerina were in progress in 1971 but do not appear to have been published in detail. Severe and repeated attacks by L. cerina on western pine species did not cause significant mortality (Staley 1978). Staley (1979) reported successful inoculations on P. ponderosa which, due to low spore density, resulted in a 2-year rather than the normal 1-year cycle. Spore germination was by an appressorium and infection by direct penetration of the cuticle.

Hendersonia acicola appears as a secondary parasite on L. cerina infected needles. Associated fungi include Ploioderma lethale (Dearn.) Darker, P. hedgcockii (Dearn.) Darker, Lophodermium australe Dearn., and two unidentified Leptostroma Fr. species (Czabator et el. 1971).

Lophodermella conjuncta (Darker) Darker.--This fungus is found only in Europe on Pinus mugo, P. nigra var. maritima, and P. sylvestris (Millar and Minter 1980).

The small, sunken, concolorous to light brown, elliptical ascocarps are on discrete browned bands of otherwise green needles, but, these bands may coalesce so that the whole needle is brown. Often the ascocarps fuse laterally. Although mature ascocarps can be found throughout the year, a peak of spore production occurs at needle flushing and new needles are infected. In Scotland, first symptoms appear as bright yellow bands, often with brown resin flecks, after 1 year (Mitchell et al. 1978). In Finland the period is 14 months (Kurkela 1978), and in Switzerland about 18 months (Terrier 1944). The period to maturation of ascocarps is 13, 24, and 19 months respectively. Thus, in Finland the fungus has a 2-year cycle. Infected needles may fall prematurely, depending upon the degree of infection and the vigour of the tree (Mitchell et al. 1978).

An epidemic of L. conjuncta occurred in southern Finland from 1967 (Kurkela 1978), but the effect on stand growth has not been reported. Susceptibility of trees varies considerably within a stand. Kurkela showed a relationship between precipitation and spore liberation and conducted successful field inoculation experiments on Scots pine.

No specific chemical control has been reported, but Mitchell et al. (1978) noticed that zineb (Dithane), applied to new foliage to control L. sulcigena, also reduced the incidence of L. conjuncta.

Hendersonia acicola, an undescribed species of Phaeoseptoria Speg., and Lophodermium staleyi Minter have been found associated with young lesions caused by L. conjuncta.

Lophodermella maureri nov. sp.--This new species has been found recently in Mexico on Pinus ayacahuite by E. S. Maurer; D. D. Skilling and I transmitted the fungus to D. W. Minter, who is describing it.

COMPARISON OF SPECIES

Geographical Distribution and Host Specificity

L. sulcigena and L. conjuncta are clearly European species on two-needled pines of the Sylvestres Subsection of the Subgenus Pinus (Critchfield and Little 1966). L. morbida, L. montivaga, L. arcuata, and L. concolor are clearly western North American species, although L. concolor spreads as far eastwards as Ontario on P. banksiana. L. cerina shows a discontinuous distribution on western and southern pines, whilst L. maureri is known only from Mexico thus far.

The two European species are restricted to European two-needled pines; the record on P. radiata is a misquote concerning L. montivaga and the report of L. sulcigena on P. contorta (Kujala 1950) never has been confirmed (Karlman 1980). L. sulcigena and L. conjuncta have infected Pinus nigra var. maritima (Corsican pine) introduced into Britain and the Netherlands although it does not appear to occur on that host in its native Corsica. It is perhaps remarkable that neither L. sulcigena nor L. conjuncta has been recorded on Pinus nigra var. nigra (Austrian pine); however, Austrian and Corsican pine are often difficult to distinguish and infections may have remained undetected. L. concolor is found only on two-needled pines, L. morbida on three-needled pines, and L. arcuata and L. maureri are restricted to five-needled pines of the Section Strobus.

L. montivaga and L. cerina appear to be the most versatile species, especially L. montivaga which appears to attack both Haploxylon and Diploxylon pines. One wonders if there might have been some confusion of fungal species here, especially with L. montivaga on P. monticola and P. flexilis since, using the key of Hunt and Ziller (1978), a specimen on a five-needled pine with ascocarps 40-50 µ could come out to L. arcuata. Darker (1932) makes the point that ascus measurements, as used in Hunt and Ziller's key, are particularly poor characters for identification. However, Shaw and Leaphart (1960) investigated a previous possible misidentification of L. arcuata as L. montivaga in Shaw (1958) and found no evidence that a mistake had been made.

It is interesting to note that L. montivaga, L. concolor, and L. cerina, which occur on two-needled pines in western U.S.A., have each been able to infect Pinus sylvestris there, whilst L. morbida and L. arcuata, from three- and two-needled pines, respectively, have not. Whilst indicating some host specificity, this also suggests that at least some of these species would pose a threat if introduced to eastern Asia. The continental climate of central and eastern U.S.A. would seem to have protected pines there from Lophodermella, and similarly climate may have prevented the spread of L. sulcigena and L. conjuncta eastwards through Eurasia on Pinus sylvestris.

Morphology and Development

The sub-hypodermal development of the ascocarps is a key characteristic of the genus Lophodermella. Gordon (1966) ascribed the five species he examined to his Type I in which intercalary elongation of the prosenchymatous hyphal elements results in the formation of pseudoparaphyses. Reexamination of L. arcuata by Thyr and Shaw (1966) confirmed this view, but Campbell and Syrop (1975) disagreed with Gordon over L. sulcigena, because they found true paraphyses. Uecker and Staley (1973) were unable to fit L. morbida (not examined by Gordon) into any of Gordon's types. Ascocarps of L. sulcigena and L. morbida develop similarly. I compared the ascocarps of L. sulcigena and L. montivaga and found no differences in their structure. Thus, this subject requires further detailed examination.

The sub-hypodermal nature of the ascocarps means that they tend to be more or less concolorous, and this has been used as a key character by Darker (1967) and Hunt and Ziller (1978). I find this misleading since, at least in L. sulcigena and, from photographs, apparently also in L. arcuata and L. morbida, the mature hysterothecia are quite conspicuous.

Ascospore size has been measured for all species and considerable variation exists. Darker (1932) found that length was a reliable criterion, but that width was unreliable due to shrinkage. On ascospore length, the eight species split into two groups with spores approximately 40-50 µ and 70-90 µ. By coincidence, L. cerina and L. conjuncta with long ascospores also have long life cycles. Measurements I made to compare L. sulcigena and L. montivaga (obtained from CMI Kew) gave means of 37 and 39 µ, respectively, for dried material. However, fresh spores of L. sulcigena were shorter by as much as 8% in one of three years measured. Thus, unless we establish the variation to be expected within and between years and compare only like material, spore size is unlikely to be a good character to distinguish all species.

Pycnidia have been recognised positively only in L. morbida and L. cerina and possibly in L. concolor. There is no apparent relation between possession of an imperfect state and life cycle length or ascospore size. One suspects that other Leptostroma states may yet be found.

Life Cycles

The Lophodermella species have life cycles more or less synchronized to sporulate at needle flushing time but show differences in the season of first symptom development:

Autumn - L. sulcigena, montivaga, morbida.
Spring - L. arcuata, concolor.
Summer - L. cerina, conjuncta.

Those that develop symptoms rapidly have relatively slow ascocarp development, whilst those that have delayed symptom development have rapid ascocarp development. The net result of this is that ascocarps mature at new needle development time.

Whilst the life cycle of most species seems to be constant, that of L. conjuncta and possibly L. cerina, which are the longest, vary considerably from 1 to 2 years according to geographical location or severity of needle infection. It is possible that the life cycle of other species might vary over the geographical range.

It is clear from all accounts that high humidity is necessary for ascospore ejection and germination. Drying out for an hour or more prevented spores of L. morbida from further germination, and this is probably the case with other species. All species examined form appressoria, and signs of infection pegs or penetration hyphae have been seen by several workers; but details of direct penetration of the cuticle have escaped detection. It seems unlikely that entry through stomata, as reported by Jalkanen et al. (1981) for L. sulcigena is the usual mode of entry.

Although the conditions for successful artificial infection are not clear, from my own work it appears that direct spore deposition from open hysterothecia is preferable to using a spore suspension. Darker (1932) and Staley (1979) succeeded with direct shedding of spores onto new foliage. Unfortunately, Lophodermella does not grow well in agar culture, even with pine needle extract added, so natural inoculum must be used. In view of the method of entry into the needle, there would seem to be little point in using any sort of hyphae as inoculum.

The site of infection by L. sulcigena was assumed by Millar (1970), using indirect evidence, to be a narrow zone just distal to the fascicular sheath on the emerging needle. This has not been confirmed although the observations of Uecker and Staley (1973) for L. morbida indicate a similar site. I consider that this might be the case for all species. The restriction of ascospore germination to the base of the growing needle may be due to changes in the epidermis and cuticle structure and microflora as the needle ages (Campbell 1972).

Those species that produce symptoms in autumn might be expected to have their infection spots distributed nonrandomly in zones corresponding to the base of the growing needle at the time of spore deposition, which is related to rain or periods of high humidity. The distribution of the early stages of spring symptoms has not been studied in sufficient detail to enable an association to be made. Summer symptoms, in the case of L. conjuncta, are distributed at random since the hyphae colonize the needle from an infected stele (Mitchell et al. 1978). It is reasonable to suppose that L. cerina will also colonize the stele as it shows a similar type of distribution of necrotic areas in bands on the needle.

Some Lophodermella species do not colonize the needle completely. It is not known whether this is a function of the fungus or the host since the same fungus appears to colonize to different extent on different hosts. For example, L. sulcigena rarely colonizes needles of Pinus nigra var. maritima completely, usually a green basal portion of 10-20 mm remains; but it will often colonize needles of P. sylvestris to the base. L. morbida, which is otherwise similar to L. sulcigena, browns needles of P. ponderosa to the base, as does L. arcuata on its four hosts. Stopping short of the base ensures that the needles stay on the tree until maturation of the ascocarps. Cohen (1967) has shown that Hypodermella laricis v. Tub. on larch disrupts the needle's normal abscission mechanism. This may be the case also in those Lophodermella species that colonize the whole needle.

Needle cast follows soon after spore release is completed in most species. Needles browned to the base, as in L. arcuata and L. morbida, do not appear to be cast significantly earlier than those which retain a green base until after sporulation, but the records are not sufficiently detailed to determine this accurately. I would expect those which retain a green base, e.g., L. sulcigena, or have the infections in bands, e.g., L. sulcigena and L. cerina, to be cast later since it takes time for the green base to be colonized by other fungi.

Darker (1932) stated that needles of P. banksiana, infected by L. concolor, are cast after 1 year at, or slightly before, maturation of the hysterothecia. Other workers have generally found only empty ascocarps, or ascocarps colonized by secondary fungi, on cast needles. Lophodermella seems to be adapted to sporulating on the tree, close to the new susceptible needle tissue, and shedding of immature ascocarps must be assumed to be due to other causes.

Damage Caused

There is now considerable evidence from a few tree species that Lophodermella species can cause considerable reduction in growth of individual trees (Harvey 1976, Mitchell et al. 1976a, Burleigh et al. 1982). But because in most cases disease is scattered and varies in intensity from year to year at least in natural stands, the effect on stand growth would appear to be small. However, L. arcuata has been recorded specifically as infecting most trees of P. lambertiana in a

plantation of 16 ha (Burleigh et al. 1982), and Staley and Bynum (1972) imply that L. morbida had killed P. ponderosa in a provenance trial over a 20-year period. L. concolor may kill branches of P. contorta and perhaps entire trees if they are otherwise debilitated (Williams 1976).

One interesting aspect of Lophodermella species is that they are not normally recorded in nurseries. However, recently I found L. sulcigena on 2-year-old seedlings and 4-year-old transplants of Pinus sylvestris in a nursery at Crathes, near Aberdeen, Scotland. Only Terrier (1944) has mentioned nursery infection previously. This is perhaps not unexpected since Lophodermella is adapted to sporulate close to new needles. We have little or no information on the distance ascospores can travel though, by analogy with Lophodermium Chev. spores, there seems little reason why they should not carry to nurseries from nearby plantations. The nearest known source to Crathes is about half a mile (750 m). We know, however, that even in areas with a high incidence of disease, often not all needles are infected; therefore, it would not be surprising if the infection fell off dramatically away from the spore source. Thus, nursery infections are likely to be a chance occurrence with no opportunity for a buildup to epidemic levels.

Chemical Control

Chemical control has rarely been attempted. Experimentally, Dithane (zineb) or Bordeaux mixture were found to effectively control L. sulcigena on individual shoots in a relatively dry summer but less so in a wet summer (Millar 1970). This is explained if sporulation is in fact restricted to wet periods and the site of infection to a small zone distal to the fascicular sheath. In such circumstances, a single spray coinciding with spore deposition would be effective, but subsequent deposits would fall on unprotected susceptible new tissue produced at the rate of a half to several millimetres a day. Thus, unless spraying could be sustained, or timed carefully to coincide with spore deposition, it would often be ineffective. Similarly, since rapid drying of leaves probably kills germinating spores (Harvey 1976), some carefully timed sprays might be superfluous. Spraying is therefore unlikely ever to be economical.

Resistance

There is considerable evidence of natural resistance to Lophodermella species in both natural stands and in plantations where, many authors have remarked, there is close proximity of heavily infected trees and apparently healthy trees. Most studies have concerned L. sulcigena on Pinus sylvestris in Scandinavia, where northern provenances have generally proved more resistant than southern provenances. Still, considerable variation has been found within even the most resistant provenances, and differences between clones have been greatest, showing that resistance is strongly inherited (Jalkanen 1982). Some possible factors affecting resistance including needle buffering capacity, pH, mineral and total phenol content of the needles, and wax formation were examined by Jalkanen et al. (1981) and Kurkela and Jalkanen (1981). Healthy trees buffered better in late summer and had a lower pH than diseased trees. Diseased trees had significantly more N, P, K, Mg, Fe, B, and Cu and less Mg than healthy trees. Resistance is lowered when pines are grown on fertile sites, due possibly to an imbalance of micro and macro nutrients.

Franc (1977) found evidence of resistance to L. concolor in Pinus contorta and proposed to include seed from open pollinated and controlled pollinated cones of resistant trees in the Lodgepole Pine Tree Improvement programme. No other work specifically on breeding for resistance to Lophodermella has been noted.

Biological Control

Biological control of Lophodermella species by secondary fungi, especially species of Hendersonia, undoubtedly occurs, but the evidence is that it might be restricted to the lower part of the crown (Mitchell et al. 1976b) where conditions are generally more humid. Hemiphacidium longisporum appears to restrict sporulation of L. concolor on P. contorta but only in a narrow coastal strip in British Columbia (Ziller and Funk 1973), where it is influenced presumably by sea fog. The authors do not describe its distribution over the tree crown. I have recently found a case where Lophodermium seditiosum has almost completely suppressed sporulation of L. sulcigena on what appears to be P. nigra var. nigra (Austrian pine), resulting in at least 2 disease-free years.

There would seem, therefore, to exist possibilities for successful artificial biological control; but the details have yet to be worked out. Hendersonia species sporulate well in agar culture so that inoculum could be produced easily. This would make a very interesting project. I have found that Hendersonia acicola conidia do not germinate on attached green needles but do so within a few hours when the needles are detached. I suspect that they would germinate readily on Lophodermella lesions.

Conclusions

A review of the research work done on individual species of Lophodermella on pines does not shed much light on their interrelationships but rather emphasises the traits they have in common. Thus, we are compelled to look in detail again at their distribution, morphology, and life cycles. Distribution itself is not a good criterion since barriers to dispersal exist. Whilst North American pine species introduced into Europe do not appear to be susceptible to European Lophodermella species, Pinus sylvestris introduced into North America has been infected by at least three of the five North American Lophodermella species.

Although Darker (1932) drew attention to the striking similarity between L. sulcigena and L. montivaga, he retained both species in his revision of the Hypodermataceae (Darker 1967). The similarities in morphology and life cycle are such that they might indeed be the same species, but not enough is known yet about L. montivaga for a conclusion to be reached.

Similarly Darker (1932) thought that L. arcuata might simply be a form of L. montivaga or L. sulcigena on five-needled pines. But, because it differs considerably from both in having delayed symptom development and different ascospores development of the Gordon Type I, it must be considered a separate species.

What is revealed by this review is that, apart from the already known similarities between L. montivaga and L. sulcigena, L. arcuata and L. concolor show remarkable similarity in their life cycle although differing markedly in their hosts. It would be interesting to check the type of ascocarp development of L. concolor which Gordon (1966) described as Type I, since L. arcuata has been confirmed as Type I. The species do, however, differ in the appearance of the mature ascocarps and in ascospore size and would appear to be distinct.

L. cerina and L. conjuncta are both relatively long-spored species which show certain similarities also in their extended life cycles. However, L. cerina produces pycnidia, which have not been found in L. conjuncta; this, and the lack of lateral fusion of its ascocarps, must be considered sufficient to separate it from L. conjuncta.

Some careful cross-inoculation tests on each continent on P. sylvestris and P. contorta, the host common to most species, could give some very interesting results.

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Lirula macrospora on Spruce in North Dakota: Occurrence, Symptoms, and Spore Release[1]

James A. Walla[2]

Abstract.--Lirula macrospora has been found on Picea glauca and P. pungens in seventeen farmstead windbreak, block, and urban landscape plantings in seven of nine counties surveyed in northeast and north-central North Dakota. Symptom development from initial discoloration of second-year needles through sporulation on fourth-year needles is reported. Time of spore release is reported and compared with precipitation and tree growth. Current activities involving control trials are discussed.

INTRODUCTION

North Dakota is in the semiarid Great Plains region in north-central United States. Trees and shrubs are planted in North Dakota for protection, beautification, and/or profit in rural and urban areas. Rural plantings are for field shelterbelts, farmstead windbreaks, livestock protection, wildlife habitat, land reclamation, road protection and screening, Christmas trees, and wood products. Urban plantings are made in boulevard, landscape, and park areas. Spruce (Picea spp.) are not native to North Dakota but are used in all the types of plantings listed above; most are in rural areas, where 400,000 to 500,000 spruce are planted each year.

Lirula needle blight has recently been found damaging white spruce (Picea glauca (Moench) Voss) and Colorado blue spruce (P. pungens Engelm.) in plantings in North Dakota (Walla 1984). Destruction of esthetic value and lower branch dieback has occurred on some severely infected trees. Lirula needle blight is caused by the fungus Lirula macrospora (Hartig) Darker. This fungus is similar to species of the more commonly known genus Lophodermium except that Lirula spp. can have longer hysterothecia (Darker 1967). A search of North American literature indicates the fungus has been found in six states of the U.S.A. and seven provinces of Canada on several species of spruce (Andrews and Erickson 1974, Conners 1967, Darker 1932, Gilman 1952, Hunt and Wright 1957, Shope 1943, USDA 1960, Walla 1984). Darker (1932) said the disease is "widely distributed in Europe." Little information was found concerning the life cycle of L. macrospora in North American or European literature and no reports of investigations on control of L. macrospora were found. Reports by Hartig (1874) and Mer (1910) gave conflicting information. Hartig indicated three types of life cycles occur, depending on climatic conditions. Basically, in type 1 the fungus sporulates on attached third-year needles; in type 2 sporulation is on attached fourth-year needles; and in type 3 sporulation is on fallen second-year needles. Mer found all three of Hartig's types, as well as intermediates, at the same site. He believed that the various 'life cycles' of Hartig depend on time of infection. This paper reports the results of current investigations on the impact, distribution, life cycle, and control of Lirula needle blight in North Dakota.

MATERIALS AND METHODS

Distribution and Damage.--Spruce plantings in nine counties in northeast and north-central North Dakota were surveyed in 1984 for Lirula needle blight to determine the present impact and distribution of the disease and to determine its source. In each county, attempts were made to examine several types of plantings (including farmstead windbreaks, field shelterbelts, urban landscape plantings, and cemetery plantings) of various ages, species, and planting density. The year trees were planted and the source of seedlings were recorded, if that information was known. Level of damage on each tree infected by L. macrospora was recorded. Trees were considered severely infected if at least 50% of an age class of needles on at least 25% of the crown had symptoms.

Symptom and Fruiting Body Development.--Beginning in September 1983, periodic observations of symptoms were made on each class of needles on six marked shoots on white spruce near Grand Forks, N. Dak. Percentage of diseased needles on each age

[1]Paper presented at the IUFRO Working Party Conference on Recent Research on Conifer Needle Diseases [Gulfport, Miss., U.S.A., October 14-18, 1984].

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class of each shoot was recorded. Presence of symptoms (needle color, black bands) and signs (pycnidia, hysterothecia) on infected needles was also recorded. Periodic collections of infected needles from this location have been made since September 1983, for use in determining the stage of development of hysterothecia (stage determined by hand-sections or squash mounts).

Ascospore Release.--Two spore traps were placed adjacent to severely infected trees near Grand Forks on October 28, 1983. Spore trap stands consisted of a wooden clip under a 30-cm-wide wooden cover that was attached to the top of a 2-m-long rod. The rod was driven into the ground for support so the top end remained 1.3 m aboveground. A clean glass microscope slide was held in the clip at a 45° angle 10 cm below the center of the cover so that rain did not fall on the slide. Spores that landed on the slides were held to the slides by the gelatinous sheath of the spores. Spore counts were taken by counting the number of spores in a 55 x 1.76 mm path across the length and a 25 x 1.76 mm path across the width of the slide. This was accomplished by scanning one path across the length and width of the slide at 100X magnification. The same location was examined on each slide. An area of 140.8 mm^2 was observed on each slide (total of 281.6 mm^2 on 2 slides). Slides were changed sporadically from October 28, 1983, to March 23, 1984, and weekly (occasionally biweekly) from March 23 to October 1, 1984. All aseptate, clavate-filiform spores with gelatinous sheaths were counted. Precipitation data were obtained from a National Weather Service climatic data substation located within 5 km of the spore traps. Total precipitation per week was recorded and compared with spore release. Current-year shoot growth was measured periodically during the 1984 growing season.

Chemical Control Tests.--Chemical control of Lirula needle blight was attempted in 1984 in a stand of spruce near Grand Forks. The tests consisted of four treatments (no spray, June application, July application, June and July application) on selected branches on five severely infected white spruce and on healthy potted 2-2 Colorado spruce seedlings. Four branches were marked on each of the five trees and treatments were randomly assigned to branches on each tree (4 treatments, 5 replications). Treatments were randomly assigned to 12 seedlings (4 treatments, 3 replications). Chlorothalonil (5 g a.i./liter water) was applied to the stand of spruce and to the potted seedlings with a hydraulic sprayer June 1 and July 10, 1984. Branches and seedlings that were not to receive fungicide during the applications were covered with plastic bags until the spray dried.

RESULTS

Distribution and Damage.--L. macrospora was found on white and/or Colorado spruce at 14 of 83 sites examined and in five of nine counties included in the survey. It was found at three other sites in two other counties prior to this survey. Thirteen of the 17 sites were farmstead windbreak plantings. Of the 17 sites, L. macrospora was found on P. pungens at 7 and on P. glauca at 15. Both hosts were present at 11 sites. At these sites, L. macrospora was found on both hosts at 5, only on P. glauca at 6, and only on P. pungens at none. In addition to white and Colorado spruce, Norway spruce (P. abies (L.) Karst.) was found at two sites during the survey; L. macrospora was not found at those sites. Damage varied from none on some trees to severe on some trees. Where severe damage was found, large differences in infection level within and between species of spruce were evident. Establishment time of infected plantings ranged from 1934 to 1967. At least 14 of the infected plantings were established before 1957 and 2 of the plantings were established in the mid-1960's; establishment time of the remaining site has not been determined.

Symptom and Fruiting Body Development.--Needle, symptom, and fruiting body development are shown in table 1. New shoots emerged in mid-May and were fully elongated by mid-June. In 1983, first symptoms (purplish-brown bands) were observed on second-year needles (1982 needles) on October 4. Similar symptoms were present on October 28. By November 16, infected needles were entirely purplish brown and black bands were seen around some needles; but no basal black bands were found. By February 9, 1984, these needles were reddish brown and remained so through May 30. The number of black bands on needles increased during this period. Pycnidia were first observed on third-year needles collected April 26, when conidia oozed out during incubation. On June 14, infected third-year needles were brown; small hysterothecia with nondifferentiated contents were visible on some needles; and black basal bands were present. Hysterothecia enlarged to apparently full length by August 7 and short paraphyses were observed in

Table 1.--Symptom and fruiting body development[1] of Lirula macrospora on needles of Picea glauca in North Dakota.

	Needle age class			
Month	Current	2nd-year	3rd-year	4th-year
Jan.	.	gr	br	9
Feb.	.	gr	br	9
Mar.	.	gr	br	9
Apr.	.	gr	br,P	10
May	gr	gr	br	10
June	gr	gr	7	10,S
July	gr	gr	7	10,S
Aug.	gr	gr	8	10,S
Sept.	gr	br	8	10,X
Oct.	gr	br	9	X
Nov.	gr	br	9	X
Dec.	gr	br	9	X

[1]gr indicates green; br indicates some shade of brown without hysterothecia; P indicates first observation of pycnidia; 7,8,9,10 indicates stage of development of hysterothecia (7--nondifferentiated fungal mass; 8--paraphyses present, no asci; 9--immature asci present; 10--apparently mature asci present; see Hartig 1874); S indicates sporulation in field; X indicates needles remain attached after sporulation period. Observations made from October 1983 to October 1984.

some hysterothecia by August 30. Third-year needles remained brown through October 1.

On October 4, 1983, infected third-year needles (1981 needles) were brown and had hysterothecia with immature asci. The color of these needles began to fade or lighten in 1984, as they became tan (May 11, May 30), grayish-tan (June 14), and then grayish tan to gray (July 12, August 23, September 14, 22, October 4). By August 23, the 1981 needles were sufficiently brittle to be easily bent or broken off. Some hysterothecia contained some mature-sized asci from April 9 to September 6, 1984.

Symptoms other than those described above were found on a few needles from July 12 to October 1, 1984. Some fourth-year needles had symptoms similar to those on third-year needles. Some third-year needles developed new symptoms at the same time second-year needles developed new symptoms in 1984. Some third- and fourth-year needles had yellow and/or brown bands on portions of needles.

Ascospore Release.--The first spore release occurred during the first week of June in 1984 (table 2). Spores were caught during some weeks from June 4 to August 27. Most of the spores were coiled; thus length could not be measured to aid in spore identification. The first major spore release was concurrent with a large amount of rain. After this, however, the number of spores trapped per week was not correlated with amount of rain. Sporulation first occurred when current-year shoots were expanding and continued after they reached full length.

Table 2.--Numbers of Lirula macrospora ascospores observed on slides exposed in an infected spruce planting.

Date slide exposure began[1]		Exposure time	Rainfall during exposure	Number of spores trapped[2]	New shoot length[3]
		(days)	(cm)		(cm)
Apr.	23	7	2.1	0	0
	30	7	0.9	0	0
May	7	7	0.2	0	0
	14	7	Trace	0	0
	21	7	1.1	0	-
	28	7	0.1	0	-
June	4	7	11.0	1,098	-
	11	7	1.1	12	8.65
	18	7	0.3	1,386	-
	25	14	4.6	501	-
July	9	7	0.1	0	8.67
	16	7	Trace	98	-
	23	7	0.1	0	-
	30	7	0.5	149	-
Aug.	6	7	4.6	52	-
	12	6	0.2	0	-
	20	8	2.0	3	8.67
	27	7	0.1	0	-

[1]Slides were exposed from Oct. 28, 1983, to Oct. 1, 1984. Spores were found only during the period shown.

[2]Each datum is the total number of spores observed on 140.6mm^2 on each of two slides.

[3]Average length of six shoots; - indicates no data recorded.

Chemical Control Tests.--New symptoms appeared in spruce plantings by September 22, 1984, on second-year needles of some branches in all treatments. No symptoms had developed by October 10 on seedlings.

DISCUSSION

Results of surveys of spruce plantings in northeast and north-central North Dakota indicate that L. macrospora is generally at a low level of incidence and damage, but the disease is distributed throughout the area and some sites have significant disease levels. Amount of damage on individual trees at sites where some trees were significantly damaged was highly variable, with some severely infected trees immediately adjacent to lightly infected or noninfected trees. The cause of these differences may be variability in resistance, as has been found in other areas (Unger 1972, Roll-Hansen[3]).

Age of infected trees and type of sites where infected trees were found are useful in determining where the fungus came from. Assuming this fungus was introduced to each site on its host, the nursery where the seedlings were produced must be considered a possible source of the fungus. Currently, it appears likely the fungus was introduced to most sites from a nursery that had sold trees for planting in farmstead windbreaks before 1957.

Studies of symptom and fruiting body development indicate that the disease fits Hartig's type 2 pattern of development (Hartig 1874); i.e., sporulation is on attached fourth-year needles. The presence of symptoms on some needles other than those expected in Hartig's type 2 pattern may indicate that multiple patterns occur, as described by Mer (1910). A few third- and fourth-year needles with symptoms similar to those on most infected second- and third-year needles, respectively, might indicate new infections on needles not infected during the first possible year, as Hartig suggested (1874). Mer (1910) suggests this delayed symptom development is a result of infections at a later time during the first possible infection year.

Hysterothecia contained mature-sized asci from April 9 to September 6; however, spores were caught only from June 4 to August 27, with the number caught tapering off after mid-June. Some hysterothecia on moistened needles collected from April 9 to September 6 opened within 48 hours of

[3]Roll-Hansen, F. 1984. Personal correspondence. Norwegian Forest Research Institute, AS-NLH, Norway. [Letter on file].

incubation in a moist chamber. Reasons for no spore release until the week of June 4 are unknown. After mid-August, many hysterothecia in the field contained contaminants; and hysterothecia with ascospores had a gray hymenium rather than the nearly white hymenium of hysterothecia observed in June.

Hartig (1874) and Mer (1910) indicated second-year needles are the first to become infected in Hartig's type 1 and 2 patterns of development. This would not be expected considering the correlation between time of new growth and sporulation in North Dakota. Appearance of symptoms on second-year needles that were presumably protected by fungicide during the periods of sporulation in 1984 indicates infection may have occurred during sporulation in 1983 on current-year needles, or that control attempts were ineffective. The absence of symptoms on exposed, nonprotected seedlings indicates either that the symptomatic second-year needles on treated branches were infected the previous year or that the potted seedlings escaped infection.

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Mycosphaerella laricina Needlecast of *Larix decidua* in the United States[1]

Thomas H. Nicholls, Marguerita A. Palmer, and Michael E. Ostry[2]

Abstract.--The distribution, life cycle, and research being conducted on a needlecast fungus recently discovered in the United States, Mycosphaerella laricina of European larch (Larix decidua), is described.

INTRODUCTION

Red pine (Pinus resinosa Ait.), jack pine (P. banksiana Lamb.), spruce (Picea spp.), and balsam fir (Abies balsamea (L.) Mill.) are the major sources of conifer fiber in the three Lake States (Minnesota, Michigan, Wisconsin). Various disease and insect problems affecting these species have prompted forest managers to seek alternative conifer species. The USDA Forest Service (1982) has projected that the nation's pulpwood requirements will be 2.4 times greater in 2030 than in 1976, and conifer fibers are predicted to be in short supply by 1990. Unless alternative conifer species can be made available for pulp use in the Lake States soon, wood shortages may restrict the growth of the region's pulp and paper industry.

Lake States forests contain enough hardwoods to significantly increase the supply of pulpwood. However, the use of hardwood pulpwood depends on having an adequate amount of conifers available (Einspahr et al. 1984). Because of the weakness of hardwood pulps, paper machines cannot operate at economic speeds unless some of the long, strong fibers of conifers are added (Einspahr et al. 1984).

Larch has been recommended as a good alternative pulpwood species in the Lake States. Preliminary comparisons between European larch (Larix decidua Mill.) and Japanese larch (L. leptolepis Gord.) plantings indicate that these species, together with their hybrids, could outgrow pine and spruce, particularly on the better Lake States forest soils (Einspahr et al. 1984). Larch would be a valuable addition to the Lake States forest because it has rapid growth, good wood properties, and excellent pulping characteristics.

A serious needlecast disease caused by Mycosphaerella laricina (Hart.) Neg., was recently reported on European larch in Wisconsin, Michigan, and Iowa by Robbins (1982), and in Iowa and Wisconsin by Patton and Spear (1983) and Ostry et al. (1982). These were the first reports of the occurrence of this disease in the United States.

The fungus caused severe defoliation of European larch and probably has the potential to cause serious growth losses if not controlled in intensively managed plantations. Forest managers must fully understand the potential danger and be able to control this disease before larch can be commercially planted in the Lake States.

The purpose of this paper is to describe the symptoms and life cycle of M. laricina in the Lake States and to describe the current research in progress by North Central Forest Experiment Station personnel and their cooperators. This research should provide the information necessary for managers to prevent or manage this needlecast in both larch plantations and nurseries. This research is essential because little is known of the biology and host range of M. laricina in the United States or of any effective control strategies that could be utilized. Overall, our research is designed to determine host range of the fungus, growth impact on the host, fungus ecology, and effective control of the disease.

DISTRIBUTION

Surveys conducted by Robbins (1982) in 63 larch stands in Iowa, Wisconsin, and Michigan found the disease in 28 of the 42 stands of European larch and in two of four blocks of hybrid larch (L. X eurolepis), but not in 14 plantings of Japanese larch. The authors also found the disease on European larch seedlings at Wilson State Nursery, Boscobel, WI.

RESEARCH

Vaseline®-coated microscope slides were used to determine timing of spore dissemination in three affected stands in Wisconsin and Iowa in 1981 and 1982. Six slides were exposed at each location and

[1]Paper presented at the IUFRO Working Party Conference on Recent Research on Conifer Needle Diseases [Gulfport, Miss., U.S.A., October 14-18, 1984].

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changed weekly. The number of spores were counted on three transects across each slide at 430X (Ostry and Nicholls 1982). Rainfall and phenology data were also recorded each week.

Ascospores, from perithecia produced in overwintered fallen needles, were dispersed during rainfalls from April through July and served as the primary inoculum for infection. Conidia were dispersed from April to October during rainfall; most were released in the latter half of the growing season. Secondary cycles caused by conidial infections occurred when environmental conditions were favorable. The first symptoms appear on foliage in June, and fruit bodies developed by July. Initially infected needles turned yellow, and lesions developed at infection sites. Needles later became necrotic. Finally, fruit bodies developed in lesions and then appeared over the entire length of infected, necrotic attached needles. Needles in the lower portion of tree crowns were affected first. When severely infected, trees would be completely defoliated by August. When this happened, a secondary needle flush occurred and these needles also became infected. All stages of symptom development could be observed from June to October.

CONTROLS

We have decided that chemical control will not be economical or practical under plantation conditions because infection occurs throughout the growing season. However, we will evaluate different fungicides on M. laricina in vitro to determine their efficacy. Promising fungicides will be tested in the field. Effective fungicides will be registered for needlecast control in forest tree nurseries where chemical control will be practical. Weed control, pruning, and site selection may also be studied to determine the impact of this fungus.

Preliminary results have shown that genetic resistance may be the most promising control strategy for M. laricina. Larch has adequate genetic diversity and hybridizes readily. Variability in needlecast susceptibility has been observed among different Larix species and European larch seed sources. We noted that Japanese larch was resistant to the disease in this area. We are identifying resistance in several larch species and trees from several seed sources by planting them adjacent to heavily infected European larch plantations and recording subsequent infection.

We will also determine the feasibility of using biotechnology techniques for identifying somaclonal larch variants resistant to M. laricina. The identification and development of germ plasm resistant to this fungus could be important for the development and promotion of larch silviculture in the Lake States. Somaclonal technology seems ideally suited to larch because larch apparently possesses good regenerative capacity from cotyledons in microculture. In vitro systems that could be used to select, screen, and multiply larch resistant or tolerant to M. laricini could develop disease-resistant larch in significantly less time than traditional breeding programs.

Although we are primarily concerned with the impact of this fungus on European larch plantations, we are also studying its impact on Siberian Larch (L. sibirica Ledeb.) and our native tamarack (L. laricina (Du Roi) K. Koch) and western larch (L. occidentalis Nutt.). Preliminary results have shown that all three species are susceptible but western larch is the most susceptible. We must obtain information on the life history and control of M. laricina so that we can develop recommendations to prevent its introduction or spread into our native western larch and tamarack stands. Our research should identify resistant larch species and trees from seed sources that can be readily and safely utilized by forest managers. This, in turn, should provide the Lake States with high-quality, needlecast-resistant larch as an alternative conifer species that can be used to help meet the industry's future fiber needs.

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Inoculations with *Cercospora pini-densiflorae* of Various Pine Species of Different Age[1]

Yasuo Suto[2]

Abstract.--Artificial inoculations with Cercospora pini-densiflorae were made on 1- to 5-year-old seedlings of 31 pine species. The results showed that relative susceptibilities in each age group could be divided into three types based on intensity of infection.

INTRODUCTION

The needle blight of pines caused by Cercospora pini-densiflorae Hori & Nambu has spread widely over Africa, Asia, and South America; where it affects many species of Pinus (Gibson 1979). Field observations indicated that the degree of infection was different among pine species and among tree ages. These differences, however, have not been sufficiently substantiated by artificial inoculation experiments. This paper presents the results of inoculations of different aged seedlings of various pine species.

MATERIALS AND METHODS

A monoconidial isolate of Cercospora pini-densiflorae was used in these experiments. Conidia of the isolate produced on potato-sucrose agar under continuous irradiation of a black-light fluorescent lamp (Suto 1982), were utilized as inoculum. The suspension containing 15×10^4 spores/ml, mixed with Tween #20 (polyoxyethylene sorbitan monolaurate) as a spreader, was sprayed on healthy seedlings in pots or in a nursery. As a control, check seedlings were atomized only with sterile distilled water. The inoculated and check seedlings were covered with polyethylene sheets and were kept under moist conditions for 2 days.

Inoculations were made on 1- to 5-year-old seedlings of 31 Pinus species. The number of seedlings tested in a single experiment was as follows:

Number	Age
20-40	1 year
10-12	2 years
5-10	3-5 years

Most of these experiments were repeated two or more times on each species of the same age.

The seedlings were examined 60 days after inoculation. The intensity of infection was observed on current-year needles and was expressed by the following classes:

0	Not infected
0.5	A trace of infection
1	Very slightly infected
2	Slightly infected (one-third of the needles infected)
3	Moderately infected (one-half of the needles infected)
4	Heavily infected (two-thirds of the needles infected)
5	Very heavily infected (all needles infected)
6	Seedlings died (this degree of infection only on the 1-year-old seedlings)

Infection index of each tree species of each seedling age was then calculated according to the formula: Infection index =

$$\frac{0n_0+0.5n_{0.5}+1n_1+2n_2+3n_3+4n_4+5n_5+6n_6}{N}$$

where N = total number of seedlings of each experiment; n_0, $n_{0.5}$, ..., n_6 = number of seedlings in each infection intensity class.

[1]Paper presented at the IUFRO Working Party Conference on Recent Research on Conifer Needle Diseases [Gulfport, Miss., U.S.A., October 14-18, 1984].

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Table 1.--Result of inoculation experiments with Cercospora pini-densiflorae on different aged seedlings of various pine species[1]

Subgenus	Section	Subsection	Species[1]	Infection index: 1-year-old seedlings	2-year-old seedlings	3- to 5-year-old seedlings
Strobus	Strobus	Cembrae	Pinus koraiensis	2.7	0.8	1.9
			P. pumila	3.0	1.8	0
		Strobi	P. strobus	4.0	5.0	1.2
			P. griffithii	1.8	1.1	0
			P. parviflora	1.6	1.6	0.8
	Parraya	Cembroides	P. edulis	0.8	1.1	0.5
		Balfourianae	P. aristata	4.4	5.0	5.0
Pinus	Ternatae	Canarienses	P. canariensis	4.5	4.0	4.0
		Pineae	P. pinea	4.0	4.7	4.0
	Pinus	Sylvestres	P. nigra	4.9	5.0*	0
			P. pinaster	3.7*	5.0*	4.0
			P. halepensis	5.4*	5.0*	5.0
			P. sylvestris	3.3	3.2	1.6
			P. densiflora	3.1	3.0	0
			P. thunbergii	3.8	4.0	0
			P. luchuensis	3.7*	4.9	0.3
			P. insularis	5.1*	4.8	2.4
		Australes	P. palustris	0.5	2.4	0
			P. taeda	1.2	1.4	0
			P. rigida	2.8	1.8	0
			P. elliottii	1.4	0.9	0
		Ponderosae	P. ponderosa	4.9	4.5	0
			P. jeffrey	4.1*	5.0	0
			P. pseudostrobus	5.1*	4.2	0
		Sabinianae	P. sabiniana	5.0	5.0	0
		Contortae	P. banksiana	4.1	4.5	0
		Oocarpae	P. radiata	5.3*	5.0*	4.4
			P. attenuata	5.7*	5.0*	0.8
			P. muricata	5.1*	5.0*	2.3
			P. patula	2.9	3.9	0
			P. oocarpa	3.5	2.5	2.3

[1]Classification of genus Pinus follows Critchfield and Little (1966).

*20% to 100% of the seedlings were killed.

RESULTS

All of the tested Pinus species were infected with Cercospora pini-densiflorae, as shown in table 1. There were significant differences in classes of infection not only among the species of Pinus but also among the seedling age groups within the same species. Relative susceptibilities in each age group were divided into the following three types:

Type I: 1-, 2-, and 3- to 5-year-old seedlings heavily infected. Pinus aristata Engelm., P. canariensis C. Smith, P. pinea L., P. pinaster Ait., P. halepensis Mill., and P. radiata D. Don.

Type II: Both 1- and 2-year-old seedlings heavily or moderately infected, but 3- to 5-year-old seedlings slightly infected or not infected at all. Pinus strobus L., P. nigra Arnold., P. sylvestris L., P. densiflora Sieb. &

Zucc., P. thunbergii Parl., P. luchuensis Mayr, P. insularis Mill., P. ponderosa Laws., P. jeffreyi Grev. & Balf., P. pseudostrobus Lindl., P. sabiniana Dougl., P. banksiana Lamb., P. attenuata Lemm., P. muricata D. Don, P. patula Schiede & Deppe, and P. oocarpa Schiede.

Type III: 1-year-old seedlings moderately or slightly infected, 2-year-old seedlings slightly infected, and 3- to 5-year-old seedlings slightly infected or not infected at all. Pinus koraiensis Sieb. & Zucc., P. pumila Regel, P. griffithii McCelland, P. parviflora Sieb. & Zucc., P. edulis Engelm., P. palustris Mill., P. taeda L., P. rigida Mill., and P. elliottii Engelm.

Relative susceptibilities of pine species to the fungus were related to the classification of Pinus according to Critchfield and Little (1966). In subgenus Strobus, five species belonged to Type III among seven tested species. In subgenus Pinus, all of the tested species of section Ternatae, subsection Australes, and subsection Ponderosae belonged to Type I, III, and II, respectively. All of the tested species of subsections Sylvestres and Oocarpae belonged to Type II, except that P. pinaster, P. halepensis, and P. radiata belonged to Type I.

The first symptom appeared 13 to 60 days after inoculation. Incubation periods were shorter in the heavily infected species than in the slightly infected ones.

DISCUSSION

According to field observations in Japan, some exotic pines, such as Pinus strobus, P. canariensis, P. nigra, P. pinaster, P. insularis, and P. radiata, are more susceptible to the fungus than the Japanese native pines, P. densiflora and P. thunbergii, while P. palustris, P. taeda, P. rigida, and P. elliottii are resistant (K. Ito, 1981; T. Ito, 1954; Kawabata 1971; Nukumizu 1956). Although in this test only 1- and 2-year-old seedlings of P. densiflora and P. thunbergii were infected with the fungus, P. pinaster, P. halepensis, P. merkussii, P. luchuensis, P. caribeau, and P. radiata were infected not only in nurseries but also in young plantations (K. Ito, 1972; Gibson 1979; Kobayashi et al. 1979; Ogimi 1969).

In this study, susceptibilities to the fungus, shown by inoculations, differed among seedling ages as well as among pine species. Relative susceptibility of pine species, therefore, should also take into account the effect of seedling age.

Relative susceptibilities to the fungus in each different age group were divided into three types according to the intensity of infection. This information would be helpful in breeding and reforestation programs with pines.

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Resistance to *Phomopsis juniperovora* in Geographic Seed Sources of *Juniperus virginiana*[1]

Glenn W. Peterson[2]

Abstract.--Juniperus virginiana is widely used in plantings in the Great Plains of the United States; however, a blight caused by the fungus Phomopsis juniperovora is a threat to nursery production of seedlings. Progenies from 86 select trees in the Great Plains were inoculated with P. juniperovora and infection was evaluated to determine the degree of resistance to this fungus among the progenies. Several of the progenies expressed resistance to this fungus; the field growth and survival of 15 of the 20 resistant progenies after 5 years were above plantation averages. Thus, the impact of this disease in nurseries likely could be reduced by use of seed collected from resistant seed sources.

INTRODUCTION

The blight caused by Phomopsis juniperovora Hahn is the most devastating disease of Juniperus virginiana L. (eastern redcedar) in nursery seedling beds in the United States (Peterson and Hodges 1982). This fungus can destroy entire beds of J. virginiana in epidemic years if fungicides are not used (Peterson 1972). New growth is especially vulnerable. To control the disease, fungicides must be applied frequently to keep new growth protected (Otta 1974, Otta et al. 1980); this is the practice in nurseries in the Great Plains (Peterson 1981). The investigation reported herein was conducted to determine if there is genetic resistance to P. juniperovora within geographic seed sources of J. virginiana which might be used to reduce the impact of Phomopsis blight in nurseries.

P. juniperovora has a wide host range within Cupressaceae (Hahn 1940, 1943). There is considerable variation in resistance to this fungus among species and among some cultivars within species (Schoeneweiss 1969, Pero and Howard 1963). However, there have been no extensive investigations of resistance to P. juniperovora among progenies from numerous and widely separated locations, for any of the species within Cupressaceae.

J. virginiana is used widely in plantings in the Great Plains of the United States. A project on "Improvement of Juniperus species for planting on the Great Plains" was initiated in 1974. This provided an opportunity to investigate resistance among geographic seed sources of J. virginiana because seeds were collected from a large number of sources within the Great Plains.

MATERIALS AND METHODS

Seeds and Seedling Production

Single-tree lots of seed from select J. virginiana trees located in the Great Plains were obtained from D. F. Van Haverbeke, coordinator of the juniper improvement project for the Forestry Committee of the Great Plains Agricultural Council.

Cones were collected in years 1973-1976 and seeds were extracted in January 1977. After drying, the seeds were stored at -16°C until needed. To start seedling production, seeds were removed from storage, stratified in moist sand at 24°C for 1 month to increase the permeability of seed coats to water, then stratified in moist sand at 5°C for 3 months to break embryo dormancy.

Seedlings were grown in cells supported in trays that held 200 cells. Seeds were sown one to a cell in a 1:1 mixture of sphagnum peat moss and vermiculite, then covered with a 6-mm layer of peat vermiculite and a thin layer of perlite.

The trays of seeded cells were placed in a greenhouse that was kept near 18°C during the germination period; after germination was complete, a temperature of 24°C was maintained. Supplemental light was used throughout the night. Lights were on 1 minute in every 15 minutes; light intensity was approximately 75 μM photons.$m^{-2}.s^{-1}$. A fertilizer containing NPK was applied to the seedlings every week, beginning 30 days after seeds were sown.

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Inoculations

Spores for inoculation were obtained from a P. juniperovora isolate obtained from a Juniperus monosperma (Englem.) Sarg. seedling in a central Oklahoma nursery. Spores were produced on a medium with these ingredients: 1.0 g KH_2PO_4; 0.5 g $MgSO_4.7H_2O$; 2.27 g asparagine; 20 g Difco Noble[3] agar; 20 g sucrose; 1 liter distilled water. Plastic petri dishes were seeded in the center with a 6-mm disc from 6-day-old cultures grown on cornmeal sucrose agar. Cultures were incubated at 24°C in the light (25 μM photons.$m^{-2}.s^{-1}$) until spore tendrils formed (ca. 2 months), then they were kept at 16°C in the dark until used for inoculum.

Spore tendrils and globules were removed from cultures with a knife, placed in a mortar containing 5 ml of distilled water, and broken up with a pestle. The resulting suspension of spores was poured into a 1-liter flask containing 100 ml of distilled water. Spore concentration was determined with a hemacytometer and then adjusted with water to obtain the desired concentration. The suspensions used for inoculation contained ca. 1.6×10^5 spores per milliliter.

Ten seedlings of each source to be inoculated were arrayed across the short dimension of the trays. Each tray contained 10 sources; 10 alternate rows in each tray were not filled. Inoculations were made by use of equipment similar to that designed for screening southern pines for resistance to the fusiform rust fungus (Laird and Phelps 1975). The trays of seedlings were moved along the 3.4-m bed of a Hytrol[3] conveyor belt at a rate of 3 m per minute. They moved beneath three modified Devilbliss[3] atomizers placed 100 cm above the conveyor belt and approximately 65 cm above the tops of the seedlings. The atomizers were spaced 30 cm apart and were directed to the sides and middle of the trays of seedlings in a staggered pattern to minimize inoculum being blown away from the seedlings. A flask containing a suspension of spores was placed in a clamp above the conveyor. The suspended spores were kept dispersed by use of a fishtank aerator. The rate of application was 30 ml of spore suspension per atomizer per minute. This rate was regulated by three flow meters, to which air was supplied at 40 psi.

The trays of seedlings were passed twice through the spray of inoculum, once in one direction and once in the opposite direction. During each test, water agar in petri dishes was exposed to the inoculum spray; germination tests were run on the spores that were deposited on the agar. Spore germination averaged 95% overall of the tests.

Inoculated seedlings were placed in ISCO[3] growth chambers containing dew chambers. The unlighted chambers were maintained at 24°C and 100% relative humidity. After 24 hours of incubation, seedlings were placed in a greenhouse with a temperature setting of 24°C; supplemental light (80 μM photons.$m^{-2}.s^{-1}$) was provided for 2 minutes each half hour from sundown to sunrise.

A series of 10 inoculations were made, each with a separate set of seedlings. In each run, 10 seedlings of each of 86 (or fewer) sources were inoculated with the same spore suspension. The separate inoculations were considered experimental blocks.

Survival and Growth in the Field

The aforementioned juniper improvement project established 14 experimental plantings in the Great Plains in 1980. These plantings contained the geographic seed sources used in this study. Data on growth and survival after 5 years in two of the Nebraska plantings were used to examine the field performance of sources indicated as resistant to P. juniperovora.

Disease Evaluation

Evaluations were made when lesion development had progressed sufficiently, usually 12 to 14 days after inoculation. Twenty-five first- and second-order branches on each seedling were examined for presence of Phomopsis blight symptoms (lesions, necrotic foliage). Only branches with new yellowish-green foliage were evaluated because older normal green foliage is resistant (Peterson 1973). The data recorded included the (1) number of infected seedlings of each source and (2) the percentage of infected branches on each seedling.

Data Analysis

Initially, the statistical significance of differences in resistance among sources was examined by using analysis of variance ($\alpha = 0.05$). Subsequent application of Duncan's multiple range test produced a characteristic result when applied to a large number of sources. Sources with extreme differences in resistance were identified, but sources with intermediate resistance were grouped with a multilayered series of overlapping designations, thereby making evaluation of patterns of resistance difficult. Toward a goal of a more interpretable analysis, the source means were partitioned into groups with similar resistance by a cluster analysis method (Scott and Knott 1974). Unlike a multiple range test which would identify pairwise differences in resistance among sources, the cluster analysis method identifies group centers in a way that maximizes between-group variation (or equivalently, minimizes within-group variation). Similar to analysis of variance, significance of a particular partition of the sources is assessed by comparing between-group versus within-group estimates of variance with an adjustment of the latter to incorporate the residual variance estimated in the initial analysis of variance. The data are clustered into successively larger numbers of groups until between- and within-group estimates of variance are

[3]The use of trade and company names is for the benefit of the reader; such use does not constitute an official endorsement or approval of any service or product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

not significantly different ($\alpha = 0.05$). The result is a nonsubjective partitioning of the sources according to their resistance, which can be evaluated for patterns of resistance. This analysis provides no information about differences between individual sources, but it does assess the significance of differences between the centers of groups of sources.

RESULTS

The cluster analysis method was used to divide the progenies into three subgroups both with respect to branch infection data and tree infection data. These groups are identified in table 1 by the letter a, b, or c following each datum. The first group, a, referred to as the

Table 1.--Infection of progenies of Juniperus virginiana inoculated with Phomopsis juniperovora.

Progenies[1]		Infected	Infected	No. of
Number	Location	branches[2]	trees[3]	tests
		------percent------		
534-1	S. Dak.	6.7 a[4]	37.0 a[4]	4
791-1	Kans.	8.0 a	50.0 b	5
1122-6	Kans.	8.2 a	38.7 a	3
1061-4	Nebr.	8.8 a	29.0 a	10
781-5	Kans.	8.9 a	60.0 b	10
771-6	Kans.	9.6 a	52.8 b	10
762-5	Kans.	11.1 a	63.2 b	10
731-1	Nebr.	11.4 a	53.6 b	10
661-3	Nebr.	11.5 a	59.0 b	10
751-1	Nebr.	11.6 a	56.0 b	10
711-3	Nebr.	12.3 a	57.0 b	10
781-1	Okla.	12.4 a	60.5 b	6
762-4	Kans.	12.6 a	61.7 b	10
841-2	Okla.	12.8 a	67.6 b	10
841-5	Okla.	13.2 a	20.0 a	1
851-1	Tex.	13.3 a	73.3 c	3
1072-1	Iowa	13.4 a	63.9 b	10
661-2	S. Dak.	13.7 a	70.0 b	4
651-2	Nebr.	13.8 a	74.5 c	4
1023-5	Nebr.	14.2 a	63.8 b	10
781-2	Kans.	14.6 b	68.0 b	10
762-8	Kans.	14.7 b	72.9 c	10
652-4	Nebr.	14.8 b	76.0 c	10
781-4	Kans.	14.9 b	73.0 c	10
741-6	Kans.	15.5 b	71.7 c	6
611-1	S. Dak.	15.6 b	76.0 c	10
751-4	Nebr.	15.9 b	56.5 b	10
752-1	Kans.	16.0 b	59.0 b	10
1071-3	Iowa	16.0 b	66.6 b	9
862-1	Tex.	16.1 b	73.3 c	6
841-4	Okla.	16.2 b	74.3 c	10
761-4	Kans.	16.3 b	72.2 b	6
1332-4	Tex.	16.5 b	70.0 b	5
652-3	Nebr.	16.8 b	63.0 b	10
711-4	Nebr.	16.8 b	66.2 b	10
1023-4	Nebr.	17.0 b	67.8 b	10
651-3	Nebr.	17.1 b	67.0 b	10
762-6	Kans.	17.3 b	79.0 c	10
1072-2	Iowa	17.7 b	66.8 b	10
762-1	Kans.	17.7 b	70.7 b	10
711-1	Nebr.	17.9 b	73.8 c	10
1021-2	Tex.	17.9 b	77.0 c	10
1072-3	Iowa	18.4 b	70.0 b	10
652-2	Nebr.	18.5 b	70.1 b	9
711-5	Nebr.	18.7 b	63.3 b	10
1191-5	Okla.	18.9 b	67.8 b	8
1061-1	Nebr.	18.9 b	80.7 c	9
1061-5	Nebr.	19.0 b	84.1 c	10
652-5	Nebr.	19.1 b	67.1 b	10
731-5	Nebr.	19.2 b	79.0 c	10
1121-5	Kans.	19.3 b	68.0 b	5
1121-4	Kans.	19.3 b	74.4 c	9
732-6	Kans.	20.0 b	75.9 c	8
1331-3	Tex.	20.7 b	79.8 c	10
1061-3	Nebr.	20.9 b	71.1 b	8
861-1	Tex.	20.9 b	80.0 c	3
741-2	Kans.	21.1 b	71.1 b	10
1023-3	Nebr.	21.3 b	76.8 c	10
752-2	Kans.	21.3 b	77.8 c	10
711-2	Nebr.	21.6 b	76.3 c	10
862-5	Tex.	21.8 b	77.0 c	10
772-1	Tex.	21.8 b	78.1 c	10
652-1	Nebr.	21.8 b	78.8 c	10
1023-6	Nebr.	22.0 b	76.0 c	10
731-2	Nebr.	22.0 b	77.3 c	10
851-3	Tex.	22.0 b	85.6 c	7
721-1	Kans.	22.6 b	86.4 c	10
841-3	Okla.	22.6 b	91.2 c	8
651-4	Nebr.	22.8 b	81.0 c	10
732-2	Kans.	23.5 c	78.6 c	10
661-4	Nebr.	24.0 c	81.6 c	10
721-3	Kans.	24.1 c	88.6 c	10
861-3	Tex.	24.3 c	90.0 c	6
761-2	Kans.	24.4 c	86.0 c	5
1023-2	Nebr.	24.6 c	68.3 b	10
661-5	Nebr.	24.7 c	80.6 c	10
752-5	Kans.	25.5 c	83.6 c	10
651-5	Nebr.	25.6 c	86.8 c	10
1122-2	Okla.	25.7 c	69.4 b	5
1332-1	Tex.	26.4 c	83.0 c	10
1331-2	Tex.	26.6 c	79.8 c	6
1072-4	Iowa	28.3 c	78.3 c	6
851-2	Tex.	28.4 c	90.4 c	10
761-3	Kans.	28.5 c	88.7 c	10
861-4	Tex.	31.1 c	95.0 c	6
732-4	Kans.	36.1 c	93.3 c	3
Mean		18.6	72.0	

[1]Progenies arrayed according to their degree of branch infection.
[2]Basis: 25 branches on each of 10 seedlings per test.
[3]Basis: 10 seedlings per test.
[4]Means with the same letter are within the same cluster as determined by cluster analysis methods.

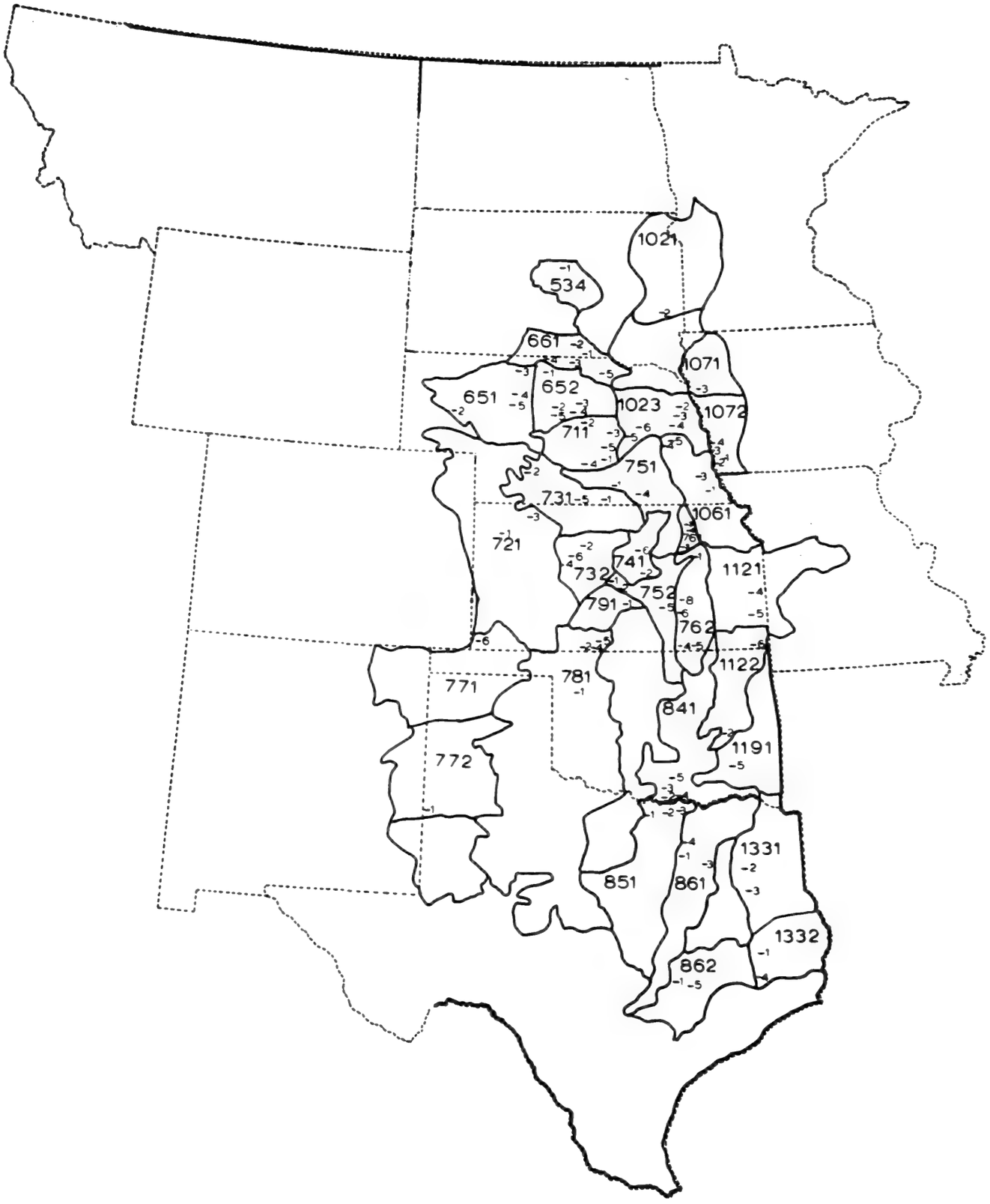

Figure 1. Location in the Great Plains of select Juniperus virginiana trees from which seeds were collected. The seed collection zones are indicated by large numbers; individual trees are indicated by small, single-digit numbers.

"resistant group," contains progenies that were most resistant to P. juniperovora. The last group, c, referred to as the "highly susceptible group," contains progenies that were least resistant. The correlation between degree of branch infection of progenies and degree of tree infection was significant ($r=.787$, $p<.001$).

The locations of the resistant (based on branch infection) progenies are not concentrated in any geographic pattern (table 1, fig. 1); they are "scattered" from north to south and from east to west. The highly susceptible progenies were also from scattered locations.

Considering all progenies, resistance was not correlated with height growth. However, most of the progenies in the resistant group were considerably above plantation averages in height growth after 5 years. Five of the resistant progenies (762-5, 791-1, 841-2, 841-5, and 851-1) were well below plantation averages; these five progenies were all from the southern Great Plains and had tops killed over winter. Survival of all progenies after 5 years was excellent; overall survival in the plantations was 96%.

DISCUSSION AND CONCLUSIONS

Information obtained on resistance to P. juniperovoa among the progenies can be utilized when establishing seed orchards of J. virginiana, when collecting seed for nursery production, and as a guide when converting the test plantings to seed orchards. Five-year growth and survival data collected from 14 test plantings established in 1980, as part of the juniper improvement project, will be published; these data will be helpful when choosing among the resistant sources. Additional information on resistance could be obtained by testing sources identified in this study in nurseries where P. juniperovora epidemics are common.

The lack of any geographic pattern of resistant sources is not surprising in that there would be no selection pressure by P. juniperovora in natural stands of J. virginiana, because this fungus seldom damages J. virginiana in natural stands. If there was selection pressure, locations where precipitation during the growing season is higher (eastern Great Plains) would have been expected to yield a higher frequency of resistant progenies.

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Some Members of the Rhytismataceae (Ascomycetes) on Conifer Needles From Central and North America[1]

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Abstract. Twenty-seven species in fourteen genera of this family, including **Hypoderma labiorum-aurantiorum** H.C.Evans & Minter, **Lophodermella maureri** Minter & Cibrián and **Naemacyclus steatopygioides** H.C.Evans & Minter, are illustrated with a brief introduction and notes. In addition **Cryocaligula** Minter gen. nov. is established for the coelomycete anamorph of Ploioderma hedgcockii.

INTRODUCTION

The ascomycete family Rhytismataceae Chev. (formerly Hypodermataceae Rehm) contains some of the most important fungal pathogens inhabiting conifer needles, including species of Cyclaneusma DiCosmo, Peredo & Minter (Naemacyclus auct.), Elytroderma Darker, Lophodermella Hohnel and Lophodermium Chev. Literature on this family is voluminous, though the coverage is uneven, most publications relating to species of Lophodermium on needles of pines. Minter (1981) has provided an extensive bibliography of these works. Two general reviews of the ecology, biology and development of species of this family have been published recently and are recommended (Minter & Cannon, 1984; Sherwood, 1981). In particular, Minter & Cannon (1984) have demonstrated the remarkable diversity of ascospore discharge mechanisms within this family, and argued that this provides further evidence that the family should be placed in an order of its own, the Rhytismatales, a long-established viewpoint (Hawksworth, Sutton & Ainsworth, 1983).

Some of the long-standing nomenclatural problems of genera within this family are now being tackled (Cannon & Minter, 1983), the taxonomy of various limited clusters of species has been revised (Cannon & Minter, 1985; Minter, 1981), and some keys are available (Darker, 1967; Hunt & Ziller, 1978; Korf, 1973; Cannon & Minter, 1985; Sherwood, 1980). Many forest pathologists still, however, rely strongly on the classic monograph of species of this family on conifer needles by Darker (1932), which is still virtually the only source of illustrations of these fungi.

Darker's (1932) monograph is, however, now becoming rather dated and hard to obtain. Furthermore, although his illustrations are generally very reliable, they suffer from certain significant limitations: they contain no information on the appearance of immature ascocarps, ascocarps in vertical section, or on anamorphs. There is thus a serious need for an up-dated collection of illustrations of these fungi, and it is the purpose of this paper to begin such a series which will provide a revision of Darker's (1932) work and augment it, if possible, where it has deficiencies.

The illustrations of each species have been produced using a standard range of magnifications, to facilitate comparisons of species. Each illustration includes: a habit sketch at life-size; a view of ascomata, conidiomata and zone lines (where present) at x 40 (i.e. as seen under a dissecting microscope); sections of ascomata and conidiomata (where present) at x 250 (i.e. at low power under a compound microscope); hymenial and conidial features at x 1000 (i.e. at high power under a compound microscope). In addition there may be extra habit sketches at x 2 and x 10 (i.e. as seen with a hand lens); extra illustrations of microscopic features (details of parts of sections, wall textures etc.) at x 250 and x 1000 as appropriate, and occasionally illustrations of critical microscopic features at x 2500 showing detail which can be discerned only with the oil-immersion lens.

Certain drawing conventions have also been used to enable the illustrations to convey more information. In drawings of sections and of highly magnified details, pigmented fungal tissue is depicted by random stippling, the concentration of dots being a rough index of the degree of pigmentation; whereas in the same drawings, dots arranged in parallel straight lines indicate that

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the pigment is non-fungal in origin, being usually tannin deposits in substrate cells. Features on the surface of asci, ascospores, paraphyses, conidia and other cells illustrated at a high magnification are drawn with a thicker pen than used for the internal features of these cells. Gelatinous deposits are indicated by a thin broken line. Guttules in, for example, young asci merely indicate that the cell has living contents, and do not necessarily accurately represent the real internal appearance of the cell so illustrated. The presence of immature asci in an illustration indicates that the species in question has asci which ripen sequentially.

Where possible each feature has been illustrated on the basis of an actual specimen. Care should therefore be employed in interpreting some of the pictures: for example the amount of tannin deposits present in the host tissues may very well vary considerably so that it is impossible to be sure given our present levels of knowledge of this group whether the presence or absence of these deposits are significant taxonomic characteristics.

These illustrations are not comprehensive: many other members of this family are known from North and Central America on conifer needles, and many more no doubt remain to be discovered. It should not therefore be assumed that a fungus of this family is new simply because it is not illustrated here, and it is recommended that these illustrations be used principally in conjunction with Hunt & Ziller's (1978) valuable keys.

For the sake of ease of layout, the illustrations are set out after the Literature Cited at the end of this paper, in alphabetical order by genus and species, with a brief caption identifying the species on each illustration. Full details of each illustration, together with brief notes on each species are set out immediately below.

DETAILS OF SPECIES ILLUSTRATED

Bifusella linearis (Peck) Höhnel, Annales Mycologici **15**: 318, 1917.
Rhytisma lineare Peck, New York State Museum Annual Report **25** (1871): 100, 1873.

Illustration: all parts drawn from IMI 241696 (North American material). A. Habit on needles. B. Detail of ascoma and a blackened sterile stromatic area. C. Ascoma in vertical transverse section. D. Asci and ascospores. E. Conidioma in vertical transverse section. F. Conidiogenous cells and conidia. G. Detail of cells of conidioma upper wall.

Notes: this is one of a small number of species with remarkable bifusiform ascospores (Minter & Cannon, 1984) and it is known only from pines in North America. Similar species: Bifusella superba Cannon & Minter (on pines from Kashmir), Bifusella pini (Dearn.) Darker and Bifusella saccata (Darker) Darker (ascospores not so constricted). Most other species with bifusiform ascospores either occur on other host genera or have asci ripening sequentially, usually also with paraphyses present.

Cyclaneusma minus (Butin) DiCosmo, Peredo & Minter, European Journal of Forest Pathology **13**: 208, 1983.
Naemacyclus minor Butin, European Journal of Forest Pathology **3**: 160, 1973.

Illustration: all parts drawn from IMI 229735 (European material). A. Habit on needles. B. Hand lens view of ascomata (open and mature on right needle, shut and old on left needle, note zone lines of Lophodermium pinastri (Schrad.: Fr.) Chev. on left needle at bottom and surrounding top ascoma, also site of former ascoma second to top). C. Detail of four ascomata (top three shut, bottom open and multilocular), also site of former ascoma (bottom). D. Ascoma in vertical transverse section. E. Detail of ascoma in vertical transverse section (note gelatinised cells at side of hymenium providing an opening and closing mechanism). F. Asci, ascospores and paraphyses.

Notes: although this species is better known as Naemacyclus minor it has unfortunately had to be redisposed in a new genus for the following nomenclatural reasons: the type species of Naemacyclus Fuckel is N. pinastri (DeLacr. in Desm.) Fuckel, now correctly known as N. fimbriatus (Schw.) DiCosmo, Peredo & Minter. Naemacyclus fimbriatus is illustrated and discussed later in this paper and is clearly not congeneric with the present species. For this reason, DiCosmo et al. (1983) erected the new genus Cyclaneusma DiCosmo, Peredo & Minter to accommodate the present species. Cyclaneusma minus is a common inhabitant of needles of many pine species, being native probably all round the northern hemisphere. It also occurs in the southern hemisphere wherever appropriate hosts have been introduced. Similar species: Cyclaneusma niveum (Pers.) DiCosmo, Peredo & Minter (slightly larger ascomata, asci and ascospores, different cultures and pine host-range). Butin (1973) described anamorphs for both C. minus and C. niveum.

Davisomycella ampla (Davis) Darker, Canadian Journal of Botany **45**: 1423, 1967.
Lophodermium pinastri var. amplum Davis, Transactions of the Wisconsin Academy of Science, Arts and Letters **18**: 252, 1918.

Illustration: all parts drawn from IMI 224277 (North American material). A. Habit on needles. B. Hand lens view of ascomata and diffuse zone lines. C. Detail of ascomata and diffuse zone line. D. Ascoma in vertical transverse section. E. Asci, ascospores and paraphyses (note empty ascus with apical hole, centre).

Notes: this species is the type of the genus, and looks sufficiently similar to Elytroderma

deformans (Weir) Darker, the type of Elytroderma Darker, to make one wonder whether Davisomycella should be reduced to synonymy with Elytroderma. There are about seven species of Davisomycella described on conifers, keyed out by Hunt & Ziller (1978). Most have not been re-examined recently, but D. ponderosae (Staley) Dubin & Staley has asci which mature sequentially, and therefore seems unlikely to be congeneric with D. ampla.

Elytroderma deformans (Weir) Darker, Contributions from the Arnold Arboretum of Harvard University 1: 63, 1932.
Hypoderma deformans Weir, Journal of Agricultural Research 6: 277, 1916.

Illustration: (North American material). A. Habit on needles (IMI 22857). B. Hand lens view of ascomata (IMI 22857). C. Detail of part of ascoma (IMI 22857). D. Ascoma in vertical transverse section (IMI 22855). E. Ascus, ascospores and paraphyses (the top two ascospores appear to be abnormally formed) (IMI 22857).

Notes: the usually one-septate ascospores in asci which ripen synchronously (Minter & Cannon, 1984) make this species readily identifiable. Elytroderma deformans causes a witch's broom disease of Pinus ponderosa and closely related species. The rather similar European species, E. torres-juanii Diamandis & Minter occurs on Pinus halepensis, P. brutia, P. pinaster and P. pinea, but does not cause witch's broom symptoms (Diamandis, 1980).

Hypoderma labiorum-aurantiorum H.C.Evans & Minter sp. nov.

Differt ab aliis huius generis speciebus a Powell (1974) acceptis quod acus incolat pinorum et quod subepidermalia habet ascomata. Holotypus: IMI 289002, in Pini oocarpae acubus, Arrayanes, Intibucá, Honduras, legit H.C.Evans, 17 Nov. 1980. Other authentic material: IMI 288991, 288992, 288993, 288994, 288995, 288996.

Illustration: all parts drawn from IMI 289002 (Central American material, holotype). A. Habit on needles. B. Detail of ascomata and conidiomata with thin black zone line. C. Ascoma in vertical transverse section. D. Detail of clypeus and lips in vertical transverse section. E. Asci, ascospores and paraphyses.

Notes: this species has many features in common with Hypoderma rubi (Pers.) DC ex Chev., the lectotype species of Hypoderma de Not., in particular the ascospores with apparently no mucous sheath, the long-stalked asci maturing sequentially and the particularly prominent hyphal bridges at the bases of the paraphyses. Ascomata of H. rubi are, however, subcuticular, with a greatly thickened central portion of the clypeus, whereas the ascomata of this species are subepidermal and not greatly thickened in their central portions, furthermore, the lips are characteristically brightly coloured, a feature not commonly observed in this family outside the genus Lophodermium. The orange colour of the lips has provided this species with its specific epithet. No other true species of Hypoderma has been recorded on needles of pine. Ascomata of this species are accompanied by both subcuticular and subepidermal conidiomata, and by thin black zone lines. It is not clear, however, from the specimens available which, if any, of these features is truly part of this species, and which are simply parts of other members of this family growing on the same needles.

Hypoderma labiorum-aurantiorum has a wide distribution in Honduras from lowland stands of P. caribaea up to the high-altitude pines. It appears to be a relatively uncommon colonizer of fallen pine needles, competing with Lophodermium spp., its ascomata usually occurring near the needle bases.

Hypodermella laricis Tubeuf, Botanisches Zentralblatt 61: 49, 1895.

Illustration: all parts drawn from Rehm Ascomyceten 1641 in Herb. K (European material). A. Habit on needles. B. Hand lens view of ascomata on needles attached to short-shoot (note diffuse zone line at needle base (arrowed)). C. Detail of ascomata and conidiomata on needles. D. Ascoma in vertical transverse section. E. Ascus, ascospores and paraphyses.

Notes: another species with synchronously ripening asci, distinct because of the four spores per ascus and the short paraphyses. The subhymenium is very restricted in extent and the asci radiate out from it exactly as in Bifusella superba.

Isthmiella faullii (Darker) Darker, Canadian Journal of Botany 45: 1420, 1967.
Bifusella faullii Darker, Contributions from the Arnold Arboretum of Harvard University 1: 19, 1932.

Illustration: (North American material). A. Habit on needles (IMI 73283). B. Hand lens view of ascomata (left four needles) and conidiomata (right four needles) (IMI 73283). C. Detail of part of ascoma (top half of the illustration, hypophyllous) and of cluster of conidiomata (bottom half of the illustration, epiphyllous) (IMI 73284). D. Ascoma in vertical transverse section (IMI 73285). E. Asci, ascospores and paraphyses (IMI 73285).

Notes: about four species of Isthmiella Darker have been described, three on Abies and one on Picea. Hunt & Ziller (1978) key them out, but they have not been re-examined recently. Collections at Kew and CMI of this and many other American species of this family are not in sufficiently good fruiting condition to enable conidia and conidiogenous cells to be examined and illustrated, and there is now a pressing need for fresh collections

of many of these fungi, at all stages in their development.

Lirula macrospora (Hartig) Darker, Canadian Journal of Botany **45**: 1422, 1967.
Hysterium macrosporum Hartig, Wichtige Krankheiten der Waldbäume p.101, 1874.

Illustration: all parts drawn from IMI 281971 (European material). A. Habit on needles. B. Hand lens view of ascomata, conidiomata and basal zone lines. C. Detail of open ascomata and cluster of conidiomata (top left). D. Ascoma in vertical transverse section. E. Asci, ascospores and paraphyses. F. Conidioma in vertical transverse section. G. Conidiogenous cells and conidia.

Notes: this is the species in which Hartig (1874) correctly observed the true nature of ascus tip opening and ascospore discharge in this family (Minter & Cannon, 1984). The only other species like it on Picea is Lirula brevispora Ziller, with ascospores about half the length. Hunt & Ziller (1978) also key out five species of this genus on Abies.

Lophodermella maureri Minter & Cibrián sp. nov.

Differt ab aliis huius generis speciebus quae acus Pinorum incolent quod habet ascomata conspicua et ascosporas uniseptatas maturis in ascis brevirostratis. Holotypus IMI 289001, in Pini ayacahuitis acubus, Mexico, legit E.Maurer, May 1983. Other authentic material: IMI 288990.

Illustration: all parts drawn from IMI 289001 (Central American material, holotype). A. Habit on needles. B. Detail of ascoma on discoloured needle portion delimited by a diffuse zone line. C. Ascoma in vertical transverse section. D. Detail of part of clypeus in vertical transverse section showing lobed hyphae invading space between degraded epidermis and cuticle. E. Asci, ascospores and paraphysis. F. Detail of ascospore emerging from ascus tip (note constriction of spore and sheath).

Notes: this new species appears to be a pathogen in young pine plantations in Mexico. Following Darker's (1967) key this species may be placed in Lophodermella Höhnel on account of its unilocular elliptical ascomata with weakly developed basal stromatic tissue and its saccate to broadly clavate asci with clavate ascospores. Also significant is the fact that its ascomata are subhypodermal. It differs from other species in this genus known from needles of pines in having conspicuous ascomata and ascospores which are already often uniseptate in mature asci. Lophodermella maureri occurs on Pinus ayacahuite at about 2300 m, and seems to attack current year's needles, the needle base remaining green. Ascomata open in mid-June and continue to discharge spores through July: experiments aimed at controlling the fungus are under way, involving spraying with Maneb using a spreader sticker during July and August. Hunt & Ziller (1978) key out seven other species of Lophodermella, all on needles of pines.

Lophodermium australe Dearness, Mycologia **18**: 242, 1926.

Illustration: all parts are drawn from IMI 247604 (North American material). A. Habit on needles. B. Hand lens view of ascomata and conidiomata. C. Detail of ascomata and conidiomata (bottom right). D. Ascoma in vertical transverse section. E. Asci, ascospores and paraphyses (note empty ascus with apical hole, centre). F. Conidioma in vertical transverse section. G. Conidiogenous cells and conidia.

Notes: this species is rather similar to, and may indeed intergrade with L. conigenum (Brunaud) Hilitzer which is described and illustrated below. The distinguishing features of L. australe are the poor development of the ascoma lower wall (it is often even absent), the depth of embedding of the ascoma (it is subcuticular in the centre, displacing about three or four host epidermal cells, and subepidermal or even subhypodermal at the sides) and the length of the asci, ascospores and conidia. Lophodermium australe seems to be a saprophyte, fruiting on the needles only after they have died from other causes, although there is one record of this species apparently causing damage to needles of Pinus spp. introduced to Hawaii (Bega et al., 1978).

Lophodermium baculiferum Mayr, Die Waldungen von Nordamerika, p.313, 1890.

Illustration: all parts drawn from IMI 142151 (North American material, neotype). A. Habit on needles. B. Hand lens view of ascomata, light (foreground) and dark (background) conidiomata and zone line. C. Detail of open ascomata (note how splits are along lines of stomata), light (left) and dark (right) conidiomata and zone line (note tendency of conidiomata to fuse laterally). D. Ascoma in vertical transverse section. E. Asci, ascospores and paraphyses. F. Conidiomata in vertical transverse section. G. Detail of surface appearance of conidiomata, showing arrangement of ostioles around host stoma, note also textura epidermoidea around perimeter of conidioma upper wall. H. Conidiogenous cells and conidia.

Notes: this species is one of a small number of this genus on Pinus which has ascomata which open along a line of stomata. Distinguish it from the others by the following features: the totally subepidermal ascomata with strongly blackened and well-developed clypeus and lower wall tissues, the large narrow cylindrical rostrate asci and the characteristically curved paraphyses tips. Nothing is known about the biology of this species except that Mayr (1890) believed it to be a strong pathogen of Pinus ponderosa and closely related hosts.

Lophodermium canberrianum Stahl ex Minter & Millar, Transactions of the British Mycological Society 71: 336, 1978.

Illustration: all parts drawn from IMI 225310 (Australasian material, authentic for the name). A. Habit on needles. B. Hand lens view of ascomata. C. Detail of ascomata. D. Ascoma in vertical transverse section. E. Asci, ascospores and paraphyses. F. Detail of part of clypeus near split, showing lips.

Notes: this species appears to be restricted to Pinus ponderosa and its close relatives. It was first described from Australia on introduced pines, where it was reported to be a pathogen (Stahl, 1966). Staley (personal communication) has, however, subsequently reported this species also to occur on the same hosts in at least three North-Western states of the U.S.A., both inland and where quasi-maritime Christmas tree plantings have been established with inland provinces of P. ponderosa. The species is distinguished from other members of this genus on pines by its totally subepidermal ascomata with poorly developed or even absent lower walls and by the apparent absence of zone lines and conidiomata (though Staley (personal communication) reports the production of copious conidia in North American strains).

Lophodermium conigenum (Brunaud) Hilitzer, Vědecké Spisy vydávané Československou Akademií Zemědělskou 3: 76, 1929.
Lophodermium pinastri forma conigenum Brunaud, Actes de la Société Linnéene de Bordeaux 42: 95, 1888.

Illustration: all parts drawn from IMI 231805 (European material, neotype). A. Habit on needles. B. Detail of mature ascoma, immature ascoma (top), four conidiomata (three fused together longitudinally) and diffuse zone line. C. Ascoma in vertical transverse section. D. Asci, ascospores and paraphyses. E. Conidioma in vertical transverse section. F. Conidiogenous cells and conidia. G. Detail of ostiole of conidioma (note small split between two epidermal cells, surrounded by pale region then an area of darkened textura angularis).

Notes: L. conigenum is a native of Europe. One or two collections which may be of this species have been made from North America, but it cannot yet be said with any confidence that it occurs in that continent. In Europe it is known to inhabit apparently healthy needles as an endophyte, fruiting on them only after they have died from other causes (Minter & Millar, 1980). The species is most commonly encountered fruiting on needles still attached to detached branches left as trash after clear-felling or brashing or resulting from snowbreak, i.e. on healthy needles which cannot continue to live. Recent experience suggests that it may cause some minor needle browning when exceptionally large amounts of inoculum are present.

Lophodermium durilabrum Darker, Contributions from the Arnold Arboretum of Harvard University 1: 87, 1932.

Illustration: all parts drawn from holotype, Herb. FH (North American material). A. Habit on needles. B. Detail of immature (right) and mature (left) ascomata (note paler central areas of ascomata). C. Ascoma in vertical transverse section. D. Detail of clypeus central portion in vertical transverse section. E. Asci, ascospores and paraphyses.

Notes: this rarely collected species seems to be restricted to a small range of haploxylon pine hosts only in the North and West of North America. It is distinguished from all other Lophodermium Chev. species on pines by having ascomata which are subhypodermal in the central region and subepidermal at the sides.

Lophodermium juniperinum (Fr.: Fr.) de Not., Giornale Botanico Italiano 2: 46, 1847.
Hysterium juniperinum Fr., Observationes Mycologicae 2: 355, 1818.

Illustration: all parts drawn from IMI 229832 (European material). A. Habit on needles. B. Detail of mature ascomata (foreground) and immature ascoma (background, top) and conidiomata (backgound, bottom). C. Ascoma in vertical transverse section. D. Asci, ascospores and paraphyses (note empty ascus with open top centre). E. Conidioma in vertical transverse section. F. Conidiogenous cells and conidia.

Notes: this is the only species of this genus so far reported from Juniperus, although at least one other, probably an undescribed new species, exists in the herbarium at CMI.

Lophodermium nanakii P.F.Cannon & Minter, Mycological Papers (in press).

Illustration: (European material). A. Habit on needles (IMI 238668). B. Hand lens view of habit on needles (IMI 238668). C. Detail of immature (upper) and mature (lower) ascomata, with conidiomata and zone line (IMI 238668). D. Ascoma in vertical transverse section (IMI 239343). E. Asci, ascospores and paraphyses (IMI 239343). F. Conidioma in vertical transverse section (IMI 238668). G. Conidiogenous cells and conidia (IMI 238668).

Notes: this species is the common litter inhabiting Lophodermium on Picea. It seems to be native right round the northern hemisphere, apparently varying little in morphology. In the literature this fungus was usually referred to as L. piceae (Fuckel) Höhnel, but this is erroneous. Type material of L. piceae was recently examined at CMI and found to contain needles of Abies sp., not of Picea sp., bearing in very small quantities a species of Lophodermium differing in external appearance and internal structure from the common

litter inhabiting species on Picea. Hunt & Ziller (1978) key out one other species of Lophodermium on Picea and five on Abies. Collections also exist of a species of Lophodermium on litter needles of Cedrus, particularly from India. This fungus is very similar to and may indeed be the same species as L. nanakii.

Lophodermium nitens Darker, Contributions from the Arnold Arboretum of Harvard University 1: 74, 1932.

Illustration: all parts drawn from IMI 216495 (North American material). A. Habit on needles. B. Detail of ascomata, conidiomata and zone line. C. Ascoma in vertical transverse section. D. Vertical transverse section of needle showing zone line traversing needle mesophyll, penetrating the endodermis and surrounding the vascular bundles. E. Asci, ascospores and paraphyses. F. Detail of conidioma surface showing ostioles and splits. G. Conidioma in vertical transverse section. H. Conidiogenous cells and conidia.

Notes: this species is one of the better defined and hence more easily recognised members of this genus on pine needles. It is a native of North America, being known only from P. strobus and P. monticola, apparently fruiting only as a saprophyte after needles have died. Distinguish it from the other similar North American species L. molitoris Minter by its totally subcuticular ascomata and because L. molitoris has been recorded only on two- and three-needle pines.

Lophodermium pinastri (Schrader: Fr.) Chev., Flore Générale des Environs de Paris 1: 436, 1826. Hysterium pinastri Schrader, Journal für die Botanik 2: 69, 1799.

Illustration: A. Habit on needles (IMI 238368, European material). B. Hand lens view of ascomata, conidiomata and zone lines (IMI 238368, European material), note exceptionally wide-open ascoma (lower centre). C. Detail of ascomata, conidiomata and zone lines (IMI 238368, European material). D. Ascoma in vertical transverse section (IMI 225061, North American material). E. Hand lens view of ascomata, conidiomata and zone lines on cone apophysis (IMI 231791, European material). F. Asci, ascospores and paraphyses (IMI 247251, European material). G. Detail of conidioma surface showing distribution of ostioles (IMI 225060, European material). H. Conidioma in vertical transverse section (IMI 231808, European material. I. Conidiogenous cells and conidia (IMI 231808, European material).

Notes: in much of the older literature L. pinastri is treated as a very variable species both in morphology and biology, and most of the older records of Lophodermium on pines are attributed to this species. Recent research (Minter et al., 1978; Minter & Millar, 1980) however has shown that this view is erroneous. Lophodermium pinastri is now regarded as a well-defined species which is probably native only to Europe, North Africa and possibly the Western parts of Asia. It seems most likely that it is an introduction to North America, having come in with its principal host tree Pinus sylvestris. Like L. conigenum, L. pinastri is known to inhabit apparently healthy needles as an endophyte, and L. pinastri fruits on these needles only after they have died of old age. The frequent black zone lines and partially subepidermal ascomata often with prominent red lips make this species readily recognisable.

It is now realised that the taxonomy of Lophodermium species on needles of pines is far more complex than was previously thought: Minter (1981) recognised sixteen species worldwide, and another two species have subsequently been described (Cannon & Minter, 1985; Minter & Sharma, 1983). Several more undescribed species, from North and Central America reside in the CMI herbarium.

Lophodermium pini-excelsae S. Ahmad, Sydowia 8: 172, 1954.

Illustration: all parts drawn from IMI 231775 (European material). A. Habit on needles. B. Detail of mature ascomata (right), immature ascoma (top left) and conidiomata (pale areas on needle, centre and lower left). C. Ascoma in vertical transverse section. D. Asci, ascospores and paraphyses. E. Detail of conidioma surface appearance. F. Conidioma in vertical transverse section. G. Conidiogenous cells and conidia.

Notes: Lophodermium pini-excelsae is a native of the North-Western Himalaya where it occurs on five-needle pines at above about 1000 m. It also occurs in Europe and North America probably as an introduction, generally though not always on the host pine with which it was introduced. It appears, however, so far not to have posed a threat to native pines of these continents. The very pale conidiomata with shorter conidia, and the ascomata with very broad perimeter lines make this species distinctive. In its native habitat, L. pini-excelsae often occurs on the same needles as L. orientale Minter, a species which has not yet been recorded outside the Indian subcontinent.

Lophodermium ravenelii Minter, Mycological Papers 147: 33, 1981.

Illustration: all parts drawn from holotype, Herb. K (North American material). A. Habit on needles. B. Detail of ascomata, conidiomata and zone line. C. Ascoma in vertical transverse section. D. Asci, ascospores and paraphyses. E. Conidioma in vertical transverse section. F. Conidiogenous cells and conidia.

Notes: there are no recent collections of this little-known species which appears to be restricted in distribution to the Southern states of the U.S.A. and, possibly Mexico. Lophodermium ravenelii is distinguished from other species of

this genus on pines by its combination of totally subepidermal ascomata which split open between lines of stomata (the splits being lined with lips) and conidiomata with remarkably long conidia.

Lophodermium seditiosum Minter, Staley & Millar, Transactions of the British Mycological Society **71**: 300, 1978.

Illustration: (European material). A. Habit on primary needles, note drooping appearance (IMI 277425). B. Hand lens view of conidiomata on primary needles (IMI 277425). C. Hand lens view of ascomata and conidiomata on cone apophysis (IMI 256615). D. Detail of ascomata and conidioma on secondary needle (IMI 247574). E. Ascoma in vertical transverse section (IMI 247574). F. Asci, ascospores and paraphyses (IMI 247574). G. Detail of conidioma surface showing ostiole (IMI 225139). H. Conidioma in vertical transverse section (IMI 225139). I. Conidiogenous cells and conidia (IMI 225139).

Notes: almost all serious outbreaks of Lophodermium needlecast on pines can be attributed to this species which appears to be native to Europe and introduced to North America. This species is usually encountered on P. sylvestris and P. nigra but may also be found on P. resinosa which is not a natural host. It is known to occur in Europe in apparently healthy needles as an endophyte. On larger and healthy trees it rarely kills the needles, and indeed is not often encountered except in forests where L. conigenum is absent, when it occupies the normal habitat of this species (Minter, 1981). Lophodermium seditiosum can however cause significant problems on young trees, especially those in the nursery and under stress. For access to the literature on control see the bibliography in Minter (1981).

Meloderma desmazieresii (Duby) Darker, Canadian Journal of Botany **45**: 1429, 1967.
Hypoderma desmazieresii Duby, Mémoires de la Société de Physique et d'Histoire Naturelle **16**: 54, 1861.

Illustration: A. Habit on needles (IMI 225837, European material). B. Detail of ascomata and conidiomata (IMI 225837, European material). C. Ascoma in vertical transverse section (IMI 225837, European material). D. Asci, ascospores and paraphyses (IMI 225837, European material). E. Conidioma in vertical transverse section (IMI 19818, North American material). F. Conidiogenous cells and conidia (IMI 19818, North American material).

Notes: two species of this genus have been described on needles of conifers, both on pines. Meloderma desmazieresii, although originally described from Europe, is probably a native of North America, introduced to Europe with its host trees. It is most commonly encountered on five-needle pines, though it sometimes also occurs on two- and three-needle species. The other species, M. sharmarum P.F.Cannon & Minter, is known only from the Himalaya, and has ascospores tending much more towards a bifusiform shape and paraphyses which often bear small branches at their apices.

Naemacyclus fimbriatus (Schweinitz) DiCosmo, Peredo & Minter, European Journal of Forest Pathology **13**: 207, 1983.
Stictis fimbriata Schweinitz, Transactions of American Philosophical Society **4**: 179, 1832.

Illustration: (European material). A. Habit on cone apophyses and scales (IMI 223383). B. Hand lens view of ascomata on cone apophysis and scale (IMI 223383). C. Hand lens view of ascomata on needles (Desm. Pl. Crypt. France **2**, 791, Herb. K). D. Detail of ascomata on cone scale (IMI 223383). E. Ascoma in vertical transverse section on cone scale (IMI 223383). F. Asci, ascospores and paraphyses (IMI 223383). G. Hand lens view of conidiomata on cone apophysis (IMI 243758). H. Conidioma in vertical transverse section (IMI 243758). I. Conidiogenous cells and conidia (IMI 243758).

Notes: this is the correct type species of Naemacyclus Fuckel. The confusion between this fungus and species now referred to Cyclaneusma occurred because N. fimbriatus is almost invariably collected only on cones whereas the two species of Cyclaneusma have only been recorded on needles. Unfortunately, the exception to these norms is the type specimen of N. pinastri (the type of Naemacyclus and a synonym of N. fimbriatus) which is on needles. This type specimen is clearly the same species as the common fungus on cones: they both have the same characteristic hairs lining the split of the clypeus, and both have typically seven-septate ascospores in asci with rostrate apices. The anamorph appears to be uncommon and rarely collected. The only species of Naemacyclus (in this correct sense) described from needles of conifers are the ones treated in the present work.

Naemacyclus steatopygioides H.C.Evans & Minter sp. sp. nov.

Differt ab aliis huius generis speciebus in pinorum acubus inventis quod ascosporas habet in fundamento inflatas. Holotypus: IMI 289003, in Pini caribaeae acubus, Cashew Hill, Belize, legit H.C.Evans, 20 Nov. 1981. Other authentic material: IMI 289004, 289005, 289006, 289007.

Illustration: all parts drawn from IMI 289003 (Central American material, holotype). A. Habit on needles. B. Detail of ascomata on needles. C. Ascoma in vertical transverse section. D. Asci, ascospores and paraphyses.

Notes: this fungus is remarkable on account of the shape of its ascospores which are long and thin, almost filiform, but with a swollen base (the feature referred to by the specific epithet). In general among the ascomycetes, ascospores are widest either at their mid-point or towards their

apices, and this is related to the manner in which they are discharged and the form of the ascus tip through which they pass during discharge (Ingold, 1954). Minter & Cannon (1984) have already pointed out that many members of the Rhytismataceae have asci and discharge mechanisms which circumvent these constraints. Ascospores of certain of these species therefore do not follow the norms for ascospore shape described by Ingold (1954): those hitherto recorded being usually bifusiform, that is with a central constriction and equally wide top and bottom portions. The ascospores of the present fungus, being widest at the base, are even more remarkable than these bifusiform spores. No such fungus was known to Ingold (1954), and a search through the main texts on ascomycetes also revealed no similar fungus, so it is possible that this is the first time this feature has been observed in the ascomycetes.

Naemacyclus steatopygioides seems to have an erratic, predominantly upland distribution in Central America. It occurs on old hanging needles, on trash and in litter.

Ploioderma hedgcockii (Dearness) Darker, Canadian Journal of Botany **45**: 1424, 1967.
Hypoderma hedgcockii Dearness, Mycologia **20**: 240, 1926.

Illustration: all parts drawn from IMI 39561 (North American material). A. Habit on needles. B. Detail of ascomata on needle. C. Ascoma in vertical transverse section. D. Asci, ascospores and paraphyses, note aborted spores in ascus. E. Conidioma in vertical transverse section. F. Conidiogenous cells and conidia.

Notes: this species has an extremely interesting distribution in Central America, apparently being confined to isolated inland stands of P. caribaea. Such stands typically occur at altitudes of 600 - 700 m above sea level where there is a transition zone from P. caribaea to P. oocarpa. In most areas the fungus is associated with a severe blight of both primary and secondary needles and it is possible that the host trees are at the upper limit of their distribution and hence predisposed to infection. Ploioderma hedgcockii may also occur therefore on P. caribaea at lower altitudes, and on P. oocarpa at higher altitudes, but not noticeably as a pathogen. Infected needles show extensive orange-brown necrotic areas. Lines of ascomata develop later but may also occur on green primary needles with no initial evidence of a necrotic reaction.

The anamorph of this species is most unusual for a member of this family. The anamorphs of most members of the Rhytismataceae produce minute usually cylindrical aseptate conidia, spermatial in function, from flask-shaped conidiogenous cells, in conidiomata which have an upper wall only one cell thick. By comparison, this anamorph produces relatively large, snowshoe shaped one-septate conidia, which appear likely (on account of their size) to have a dispersal or survival function. Its conidiogenous cells are often swollen in their upper part, a most unusual feature among the coelomycetes in general, and its conidiomata have upper wall many cells thick. There appears to be no genus which will accommodate such a fungus. It is therefore placed in the new genus described forthwith.

Cryocaligula Minter, gen. nov. coelomycetum

Differt ab aliis generibus anamorphicis Rhytismatacearum quod habet conidia uniseptata in forma caligularum nivalium visu, cellulis e conidiogenis in apicibus incrassatis producta, et quod habet parietem superiorem conidiomatis e stratis cellularum conpluribus constructum (vide picturam). Conidiorum initium est holoblasticum. Maturatio synchrona et sequens. Secessio schizolytica et cellulae conidiogenae proliferatio sympodialis et holoblastica est ante quam secedit conidium anterior. Species typica: **Cryocaligula hedgcockii** (Dearness) Minter, comb. Basionym: Leptostroma hedgcockii Dearness, Mycologia **20**: 240, 1928.

Ploioderma lethale (Dearness) Darker, Canadian Journal of Botany **45**: 1424, 1967.
Hypoderma lethale Dearness, Mycologia **18**: 241, 1926.

Illustration: (North American material). A. Habit on needles (IMI 225838). B. Hand lens view of ascomata and conidiomata on needles (IMI 225838). C. Detail of ascomata and conidiomata on needle (IMI 225838). D. Ascoma in vertical transverse section (IMI 225838). E. Asci, ascospores and paraphyses (IMI 225838). F. Conidioma in vertical transverse section (IMI 223394).

Notes: Hunt & Ziller (1978) key out four species of this genus, all on needles of pines, using numbers of ascospores per ascus and ascospore shape and size as the main criteria. Ploioderma pedatum (Darker) Darker and P. lowei Czabator both have bifusiform ascospores. Ploioderma lethale and P. hedgcockii, which do not have such ascospores, are distinguished by their anamorphs and because P. hedgcockii has only four ascospores per ascus. More species of this genus on needles of pines collected during recent expeditions to Central America reside as yet undescribed in the herbarium at the CMI, and a new species on needles of Cedrus from India will be described shortly (Cannon & Minter, 1985).

Soleella striiformis (Darker) Darker, Canadian Journal of Botany **45**: 1427, 1967.
Bifusella striiformis Darker, Contributions from the Arnold Arboretum of Harvard University **1**: 23, 1932.

Illustration: all parts drawn from DAOM 3168 (North American material). A. Habit on needles. B. Detail of ascomata on needle. C. Ascoma in vertical transverse section (note how the hymenium

has occupied the degraded substomatal cavity). D. Asci, ascospores and paraphysis (note the 'trifusiform' ascospore).

Notes: this is the only species of this genus recorded from needles of pines. It may be readily recognised by its combination of bifusiform or even sometimes trifusiform ascospores in sequentially ripening asci in ascomata which split open along a line of needle stomata and which have no blackened lower wall. Hunt & Ziller (1978) recorded one other species, S. cunninghamiae Saho & Zinno, on needles of Cunninghamia.

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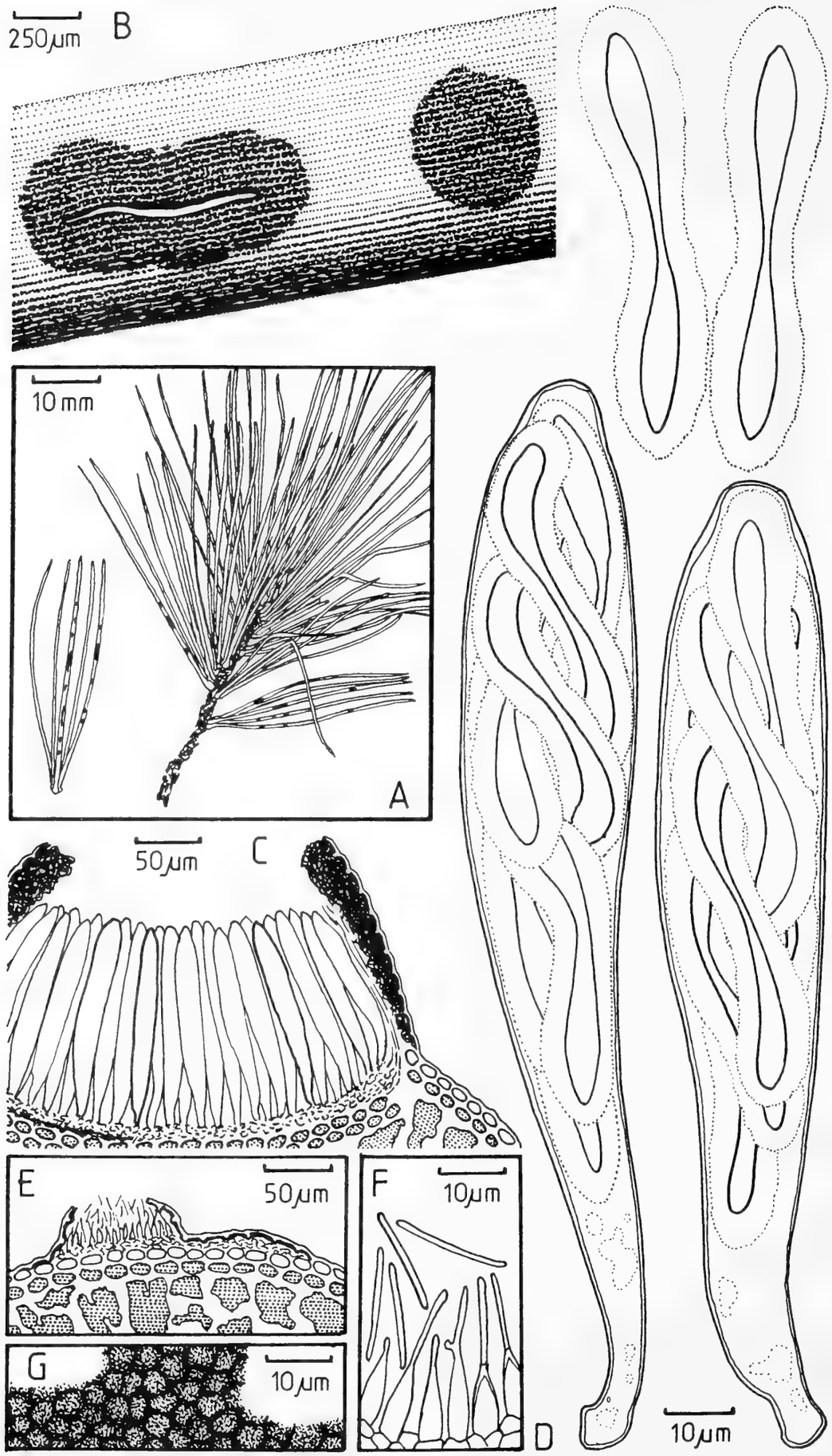
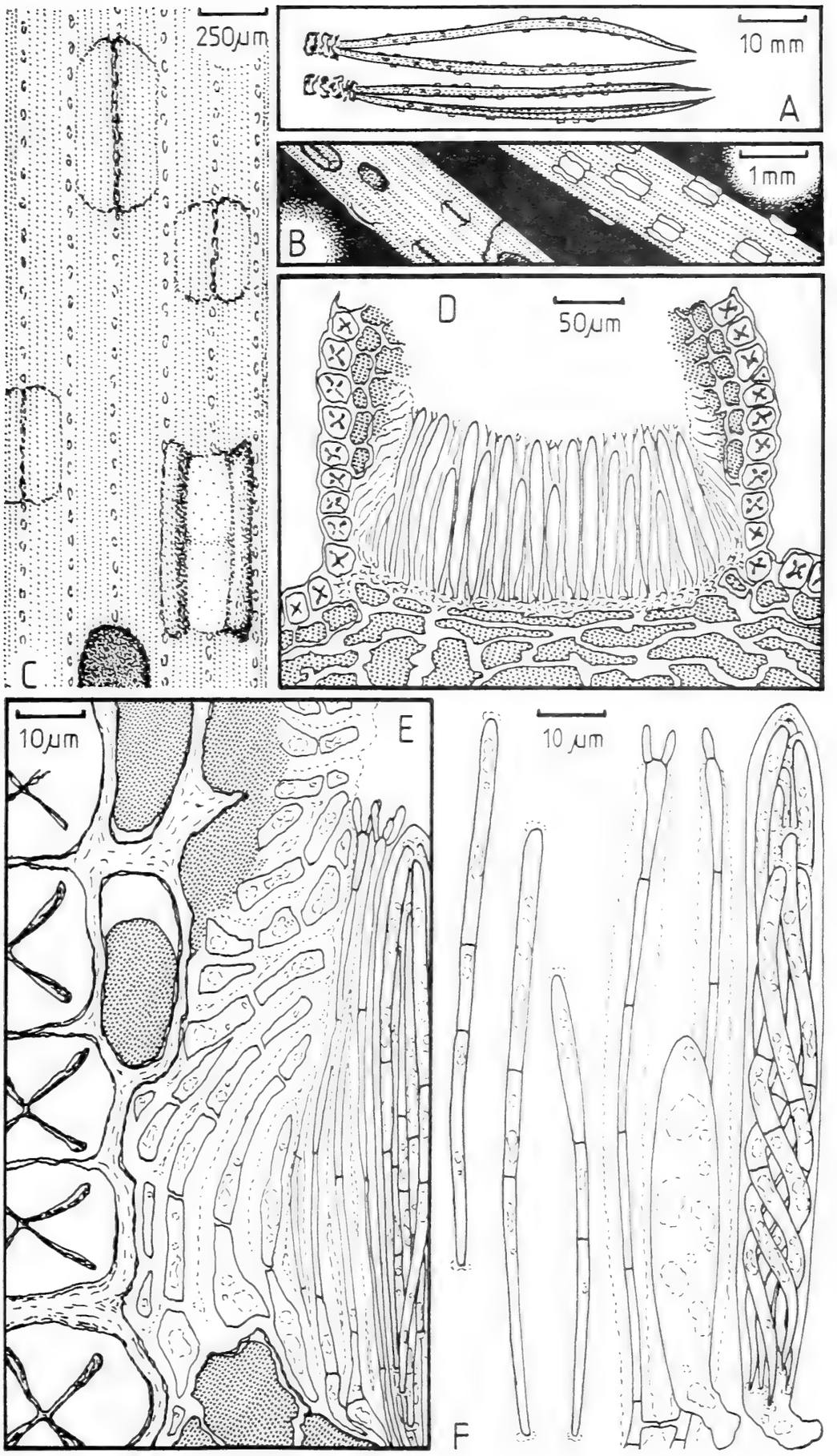
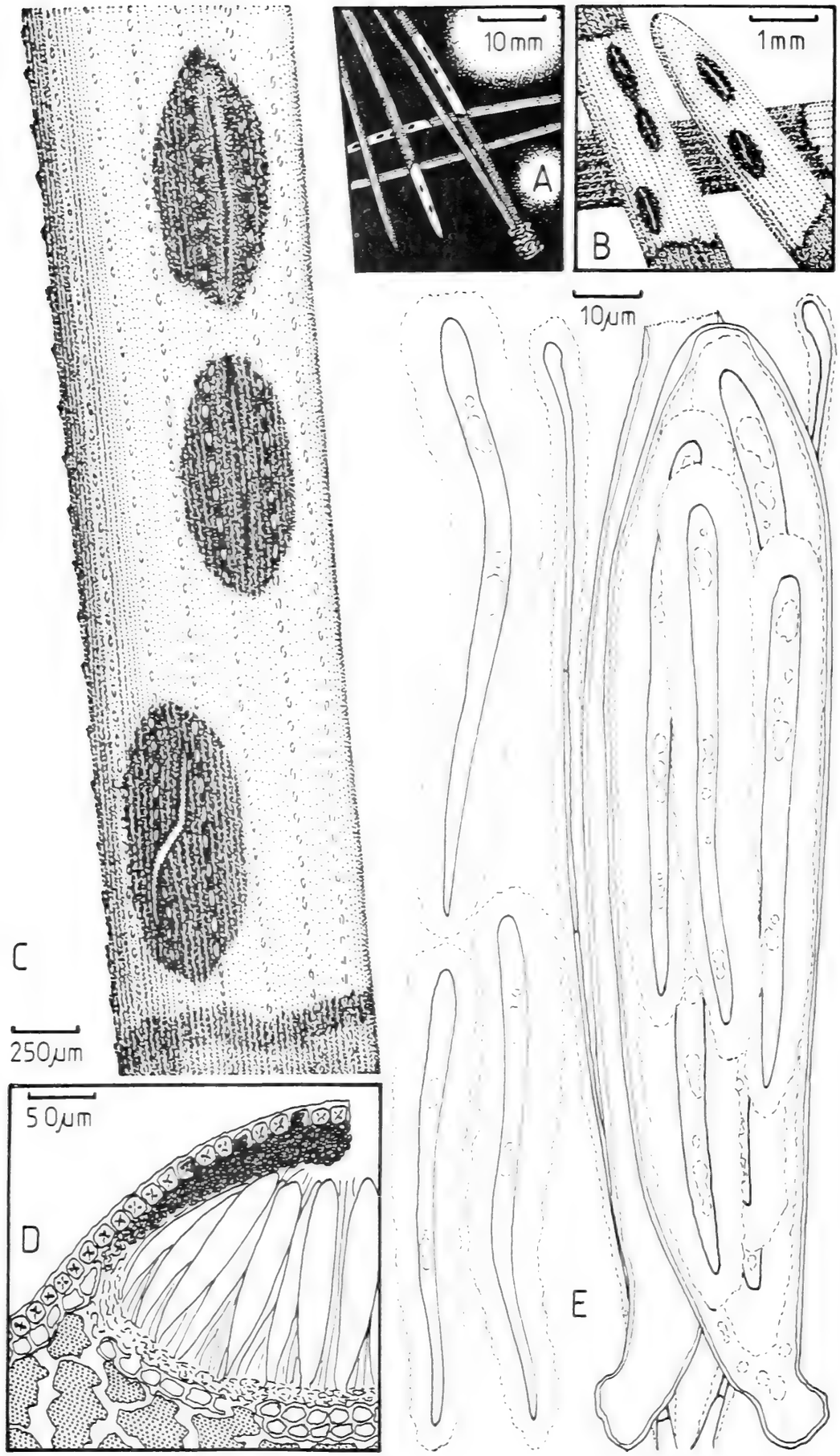


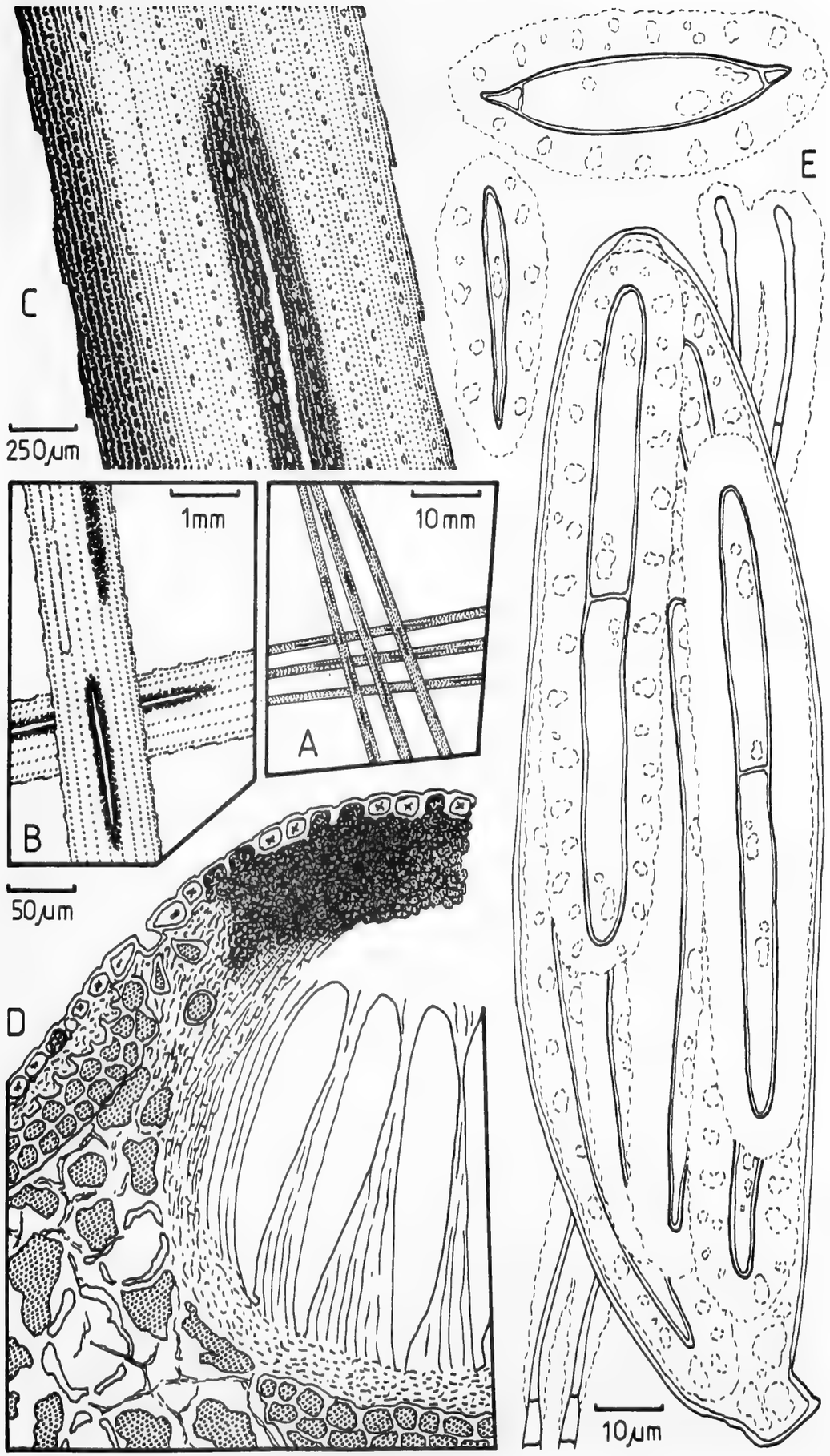
Bifusella linearis



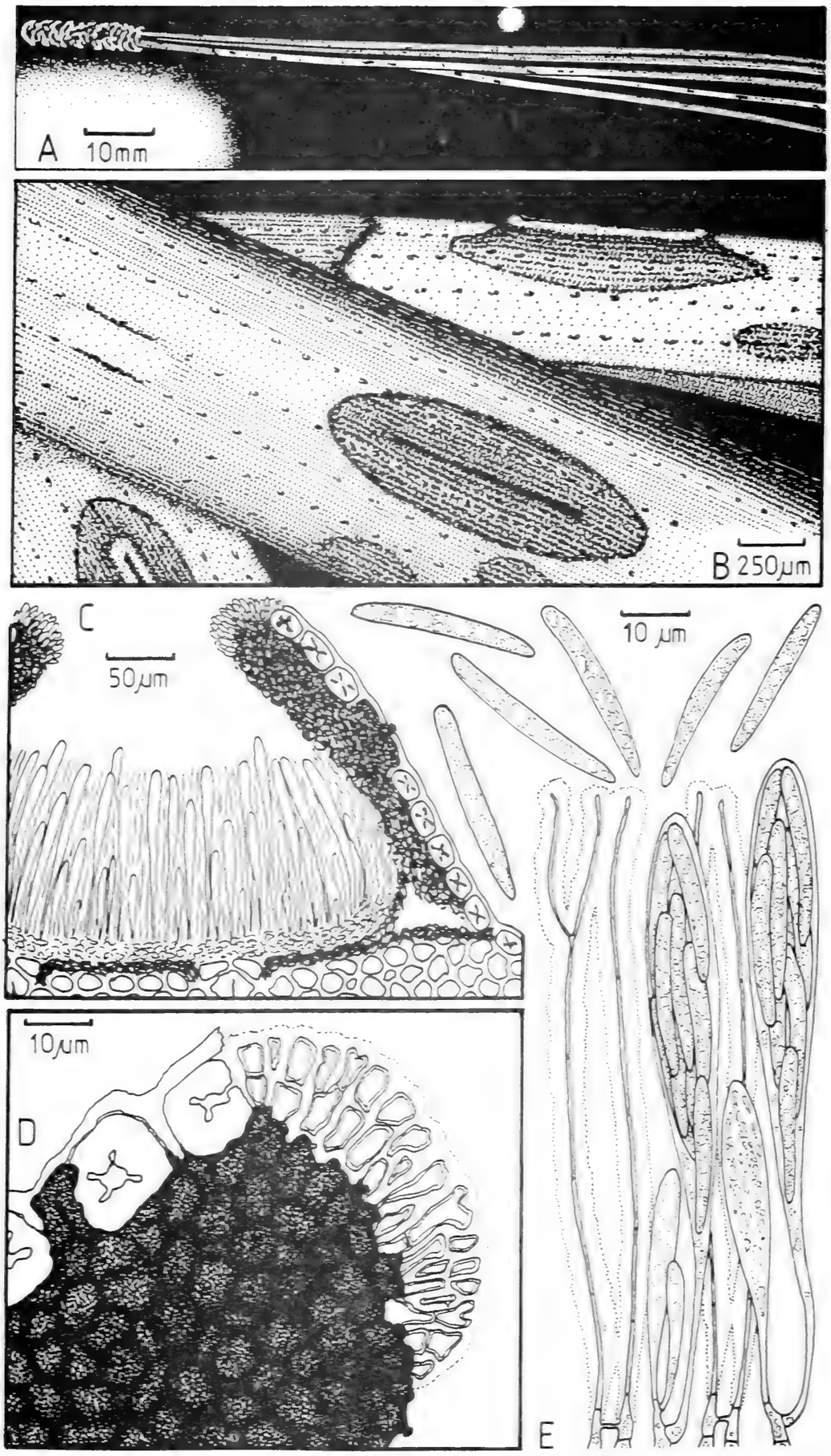
Cyclaneusma minus



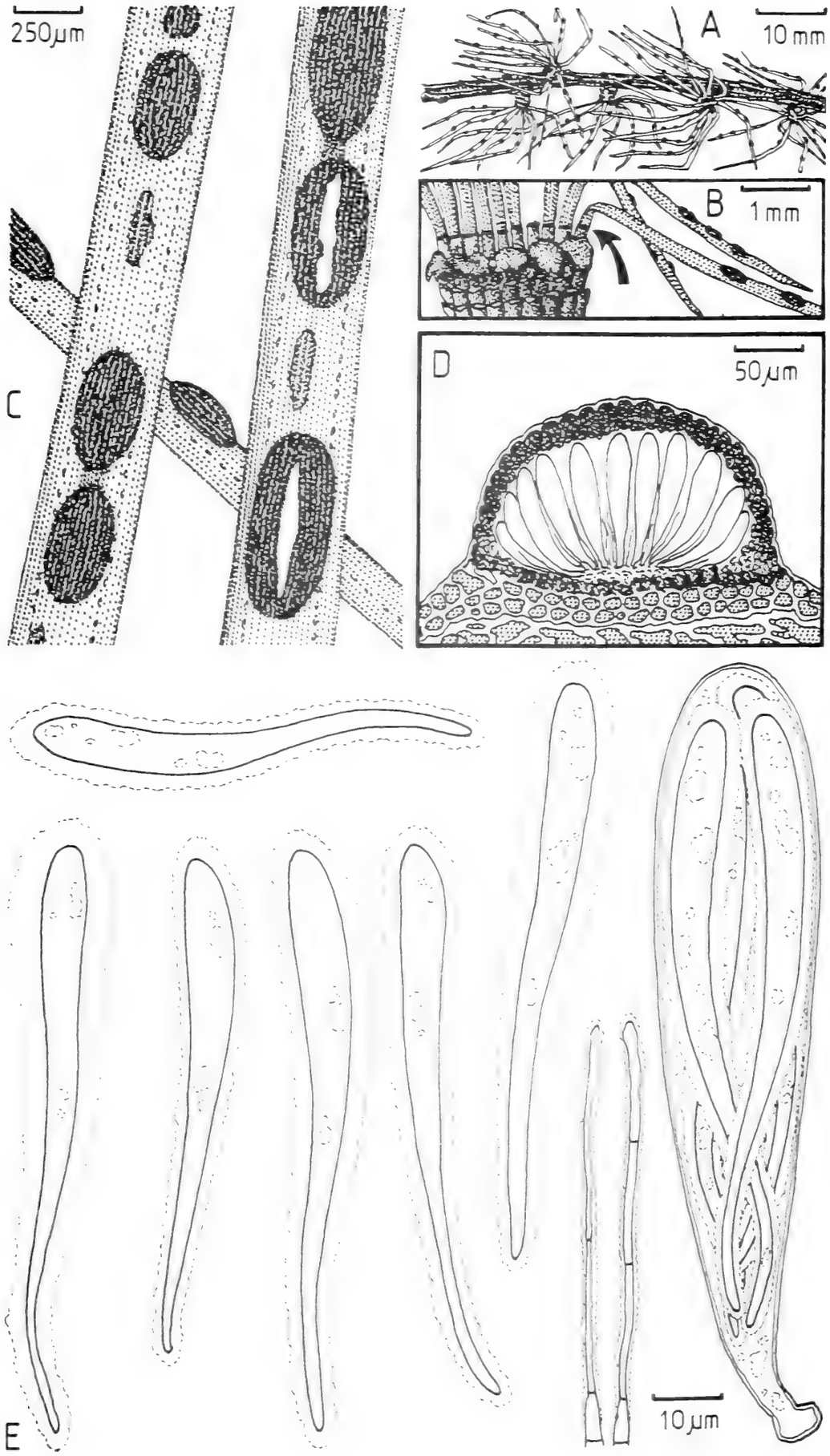
Davisomycella ampla



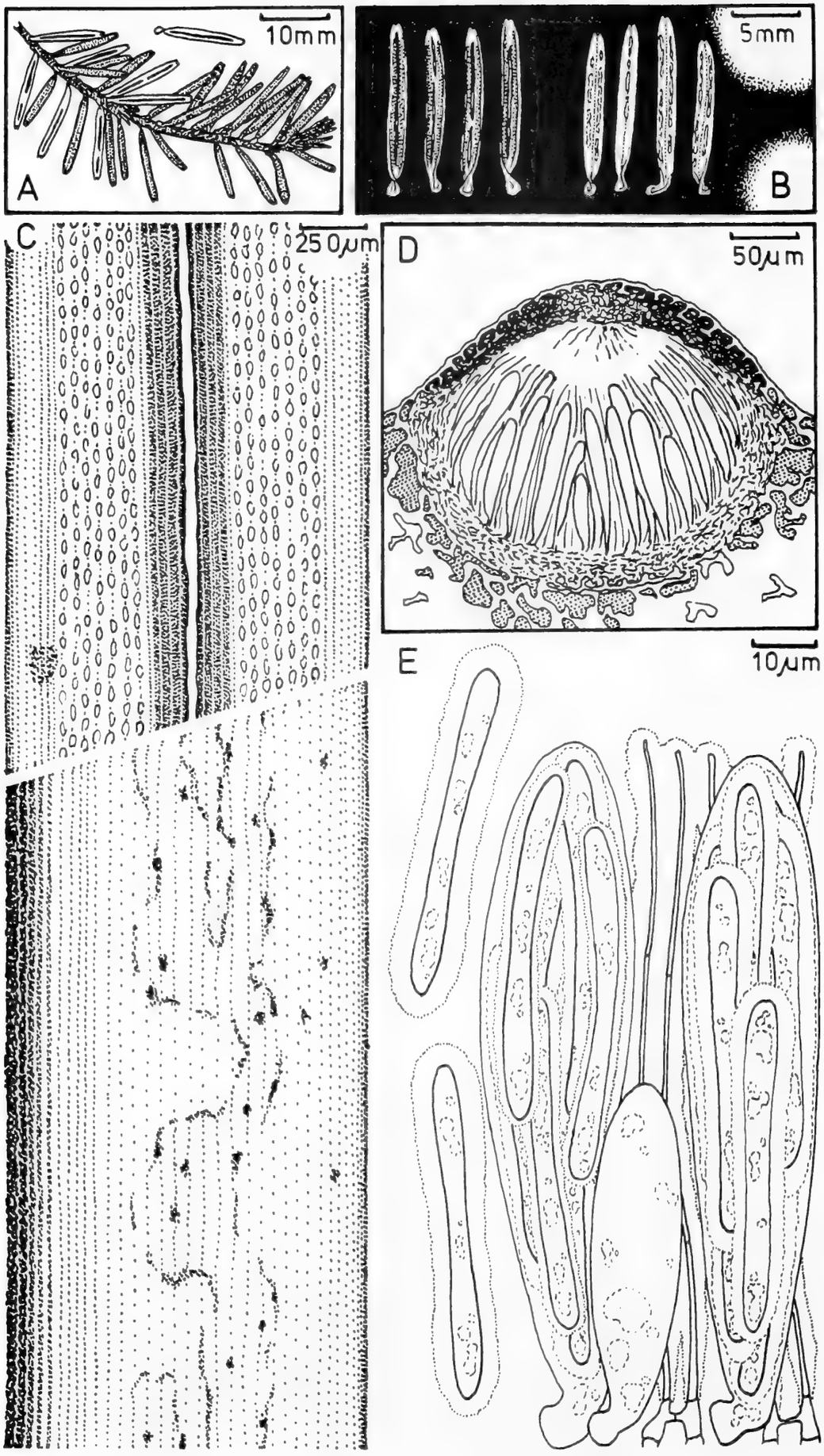
Elytroderma deformans



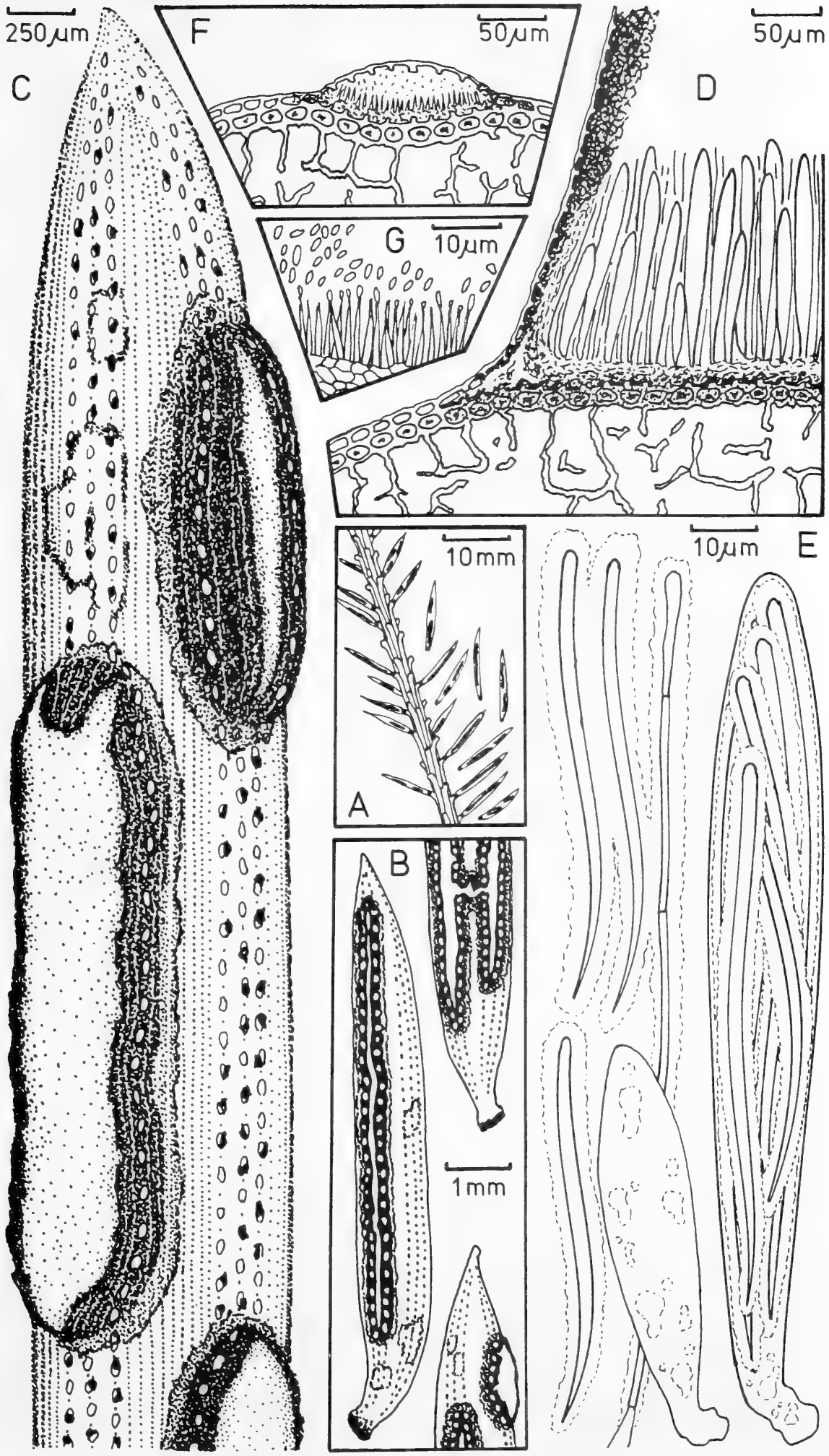
Hypoderma labiorum-aurantiorum



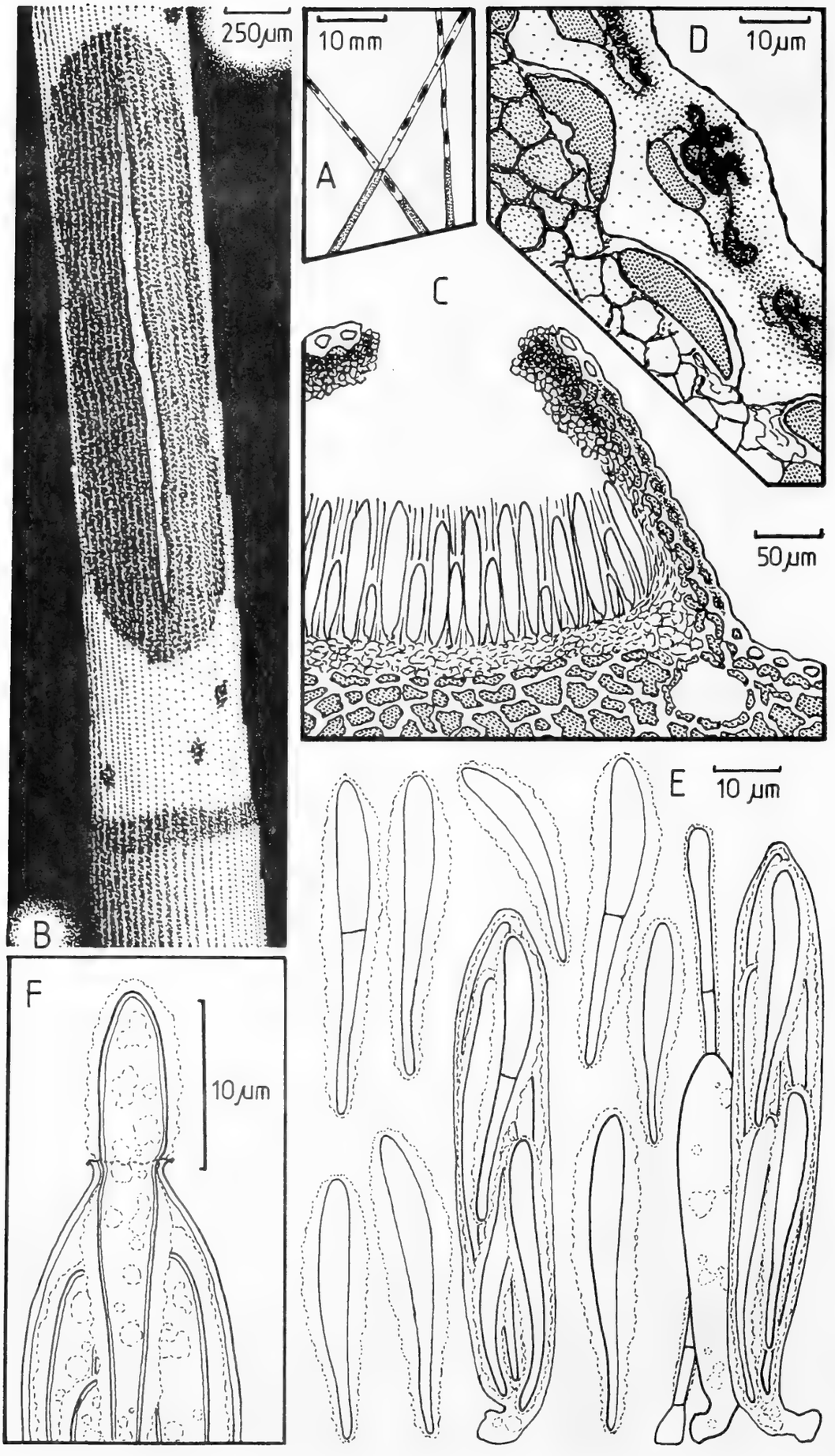
Hypodermella laricis



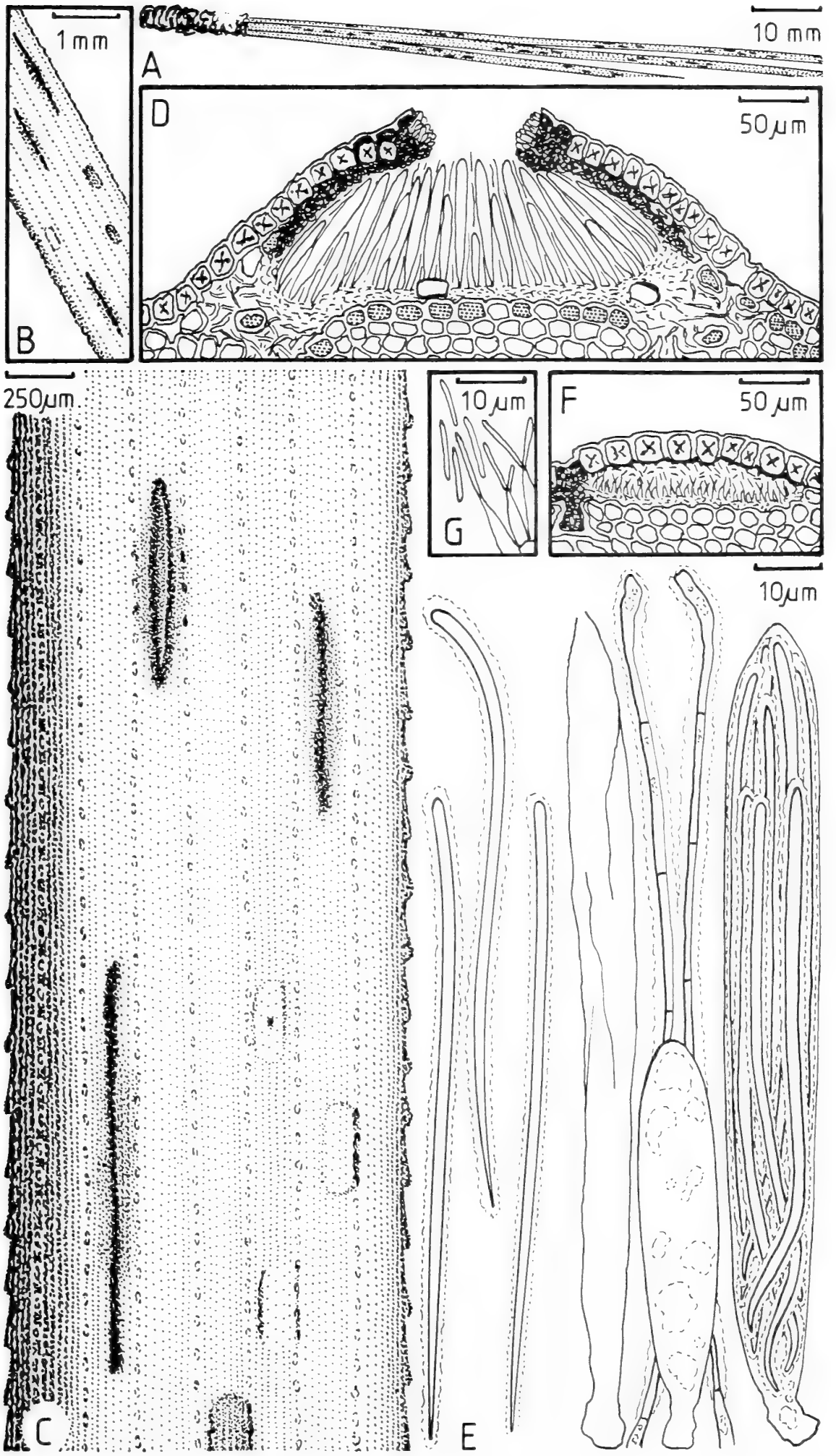
Isthmiella faullii



Lirula macrospora



Lophodermella maureri



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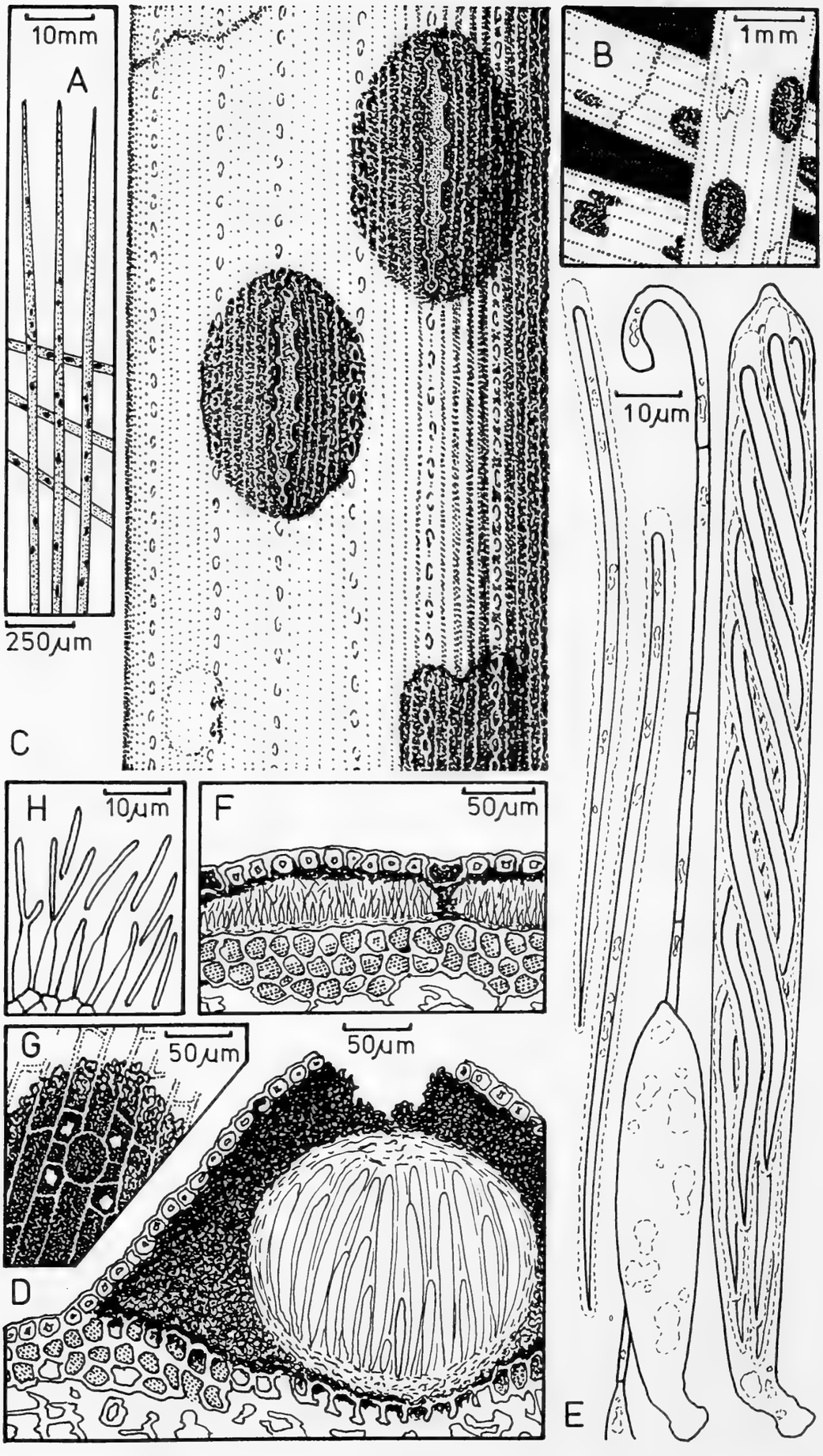
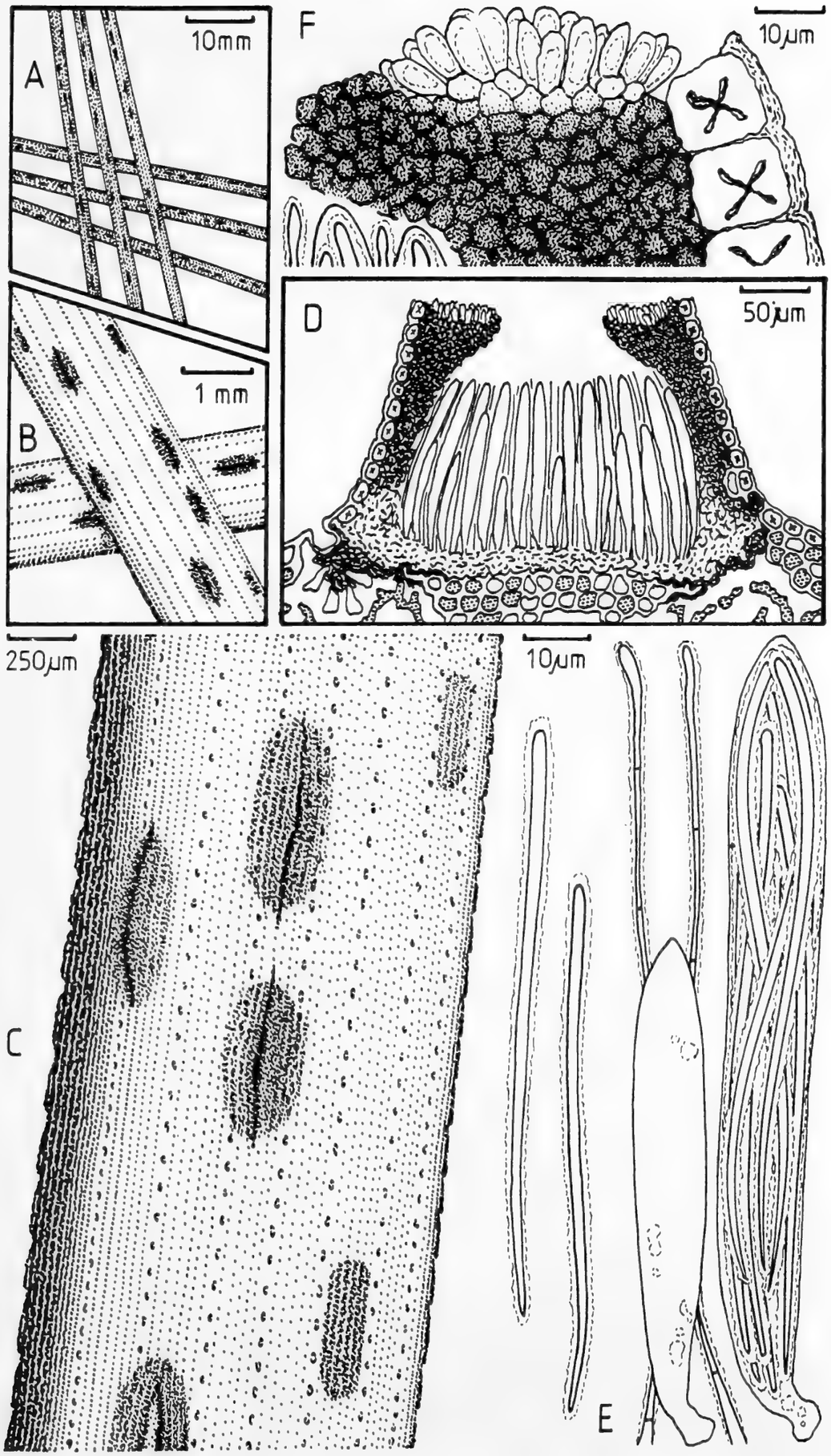
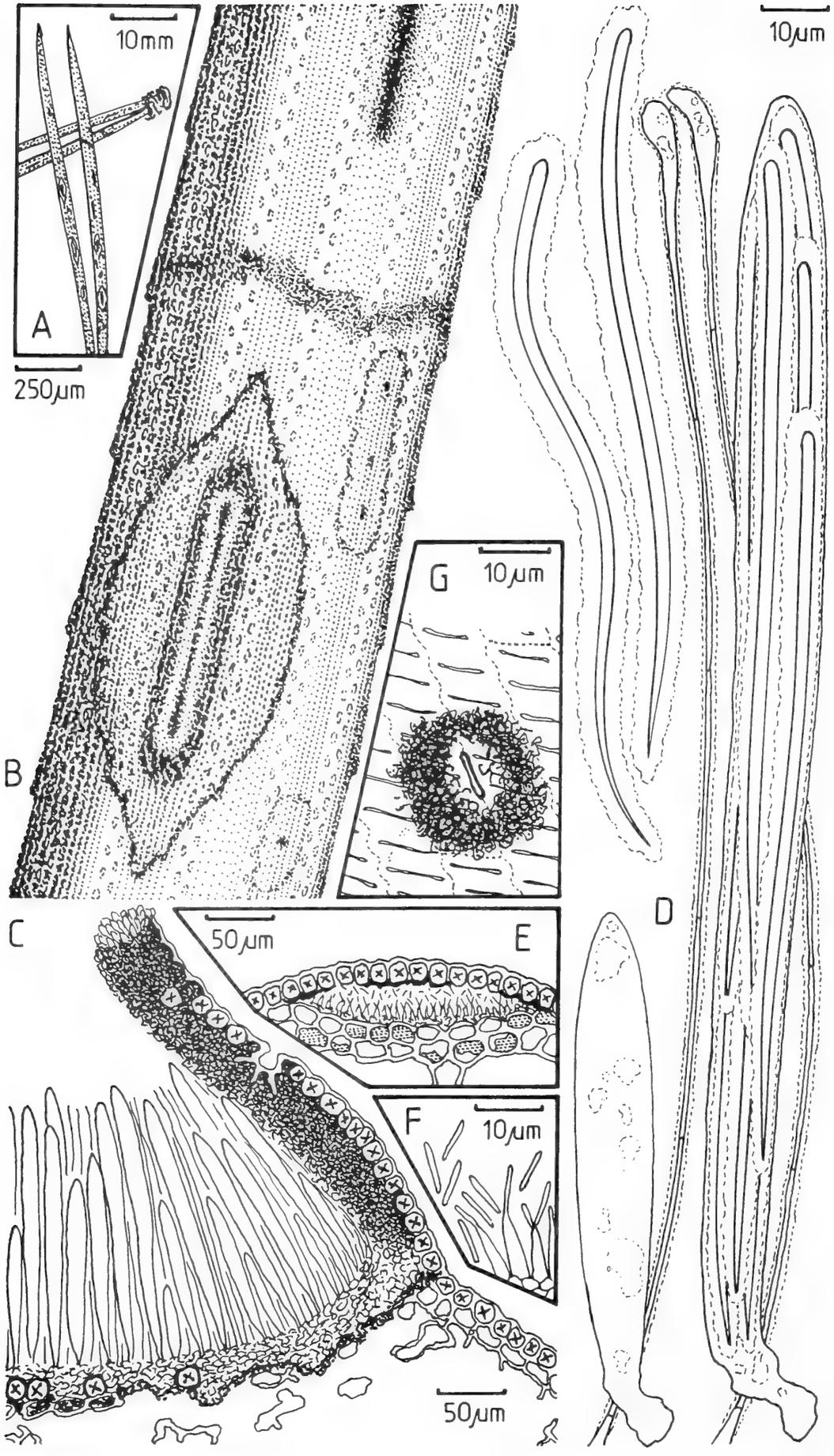


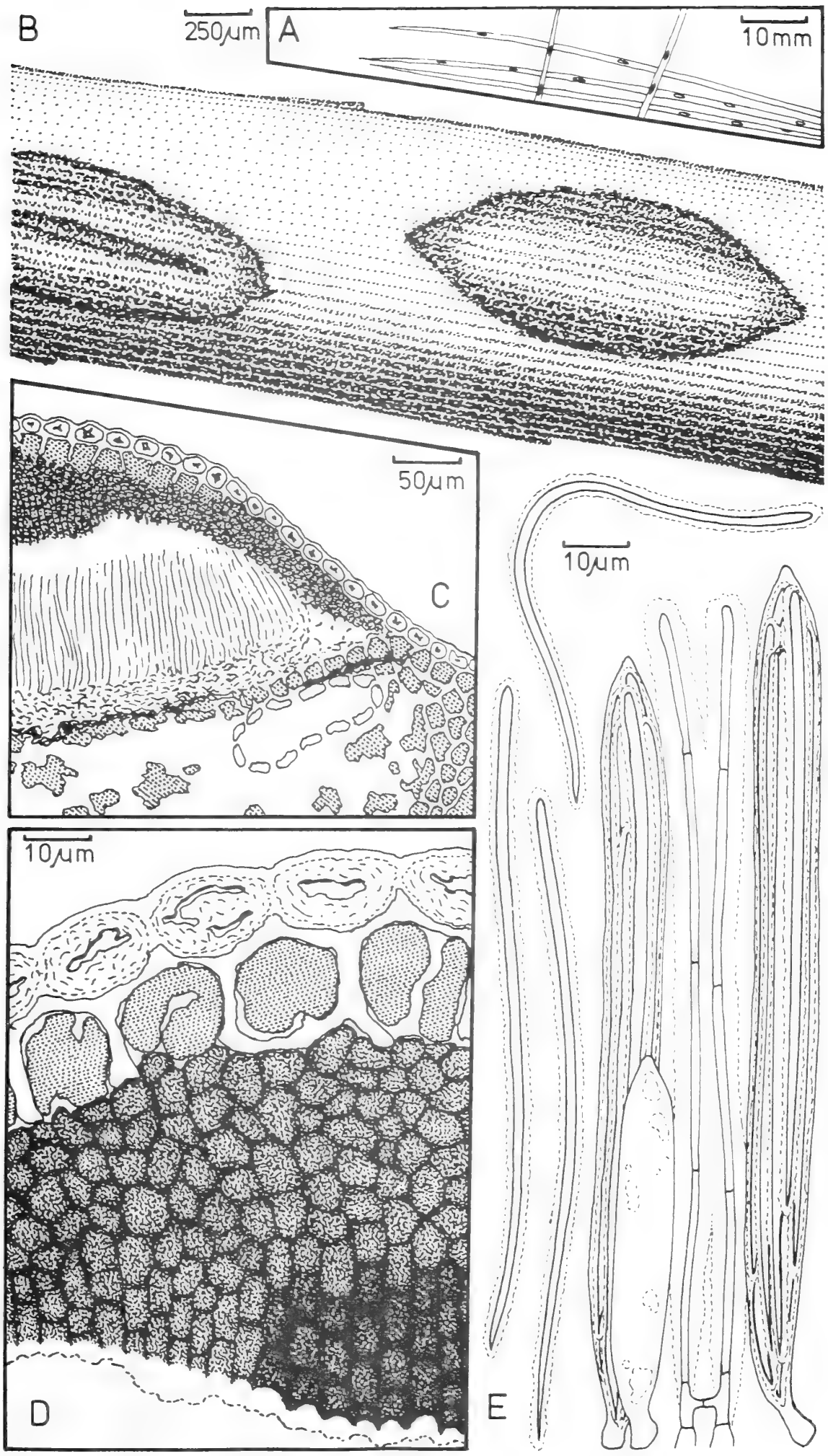
Lophodermium baculiferum



Lophodermium canberrianum



Lophodermium conigenum



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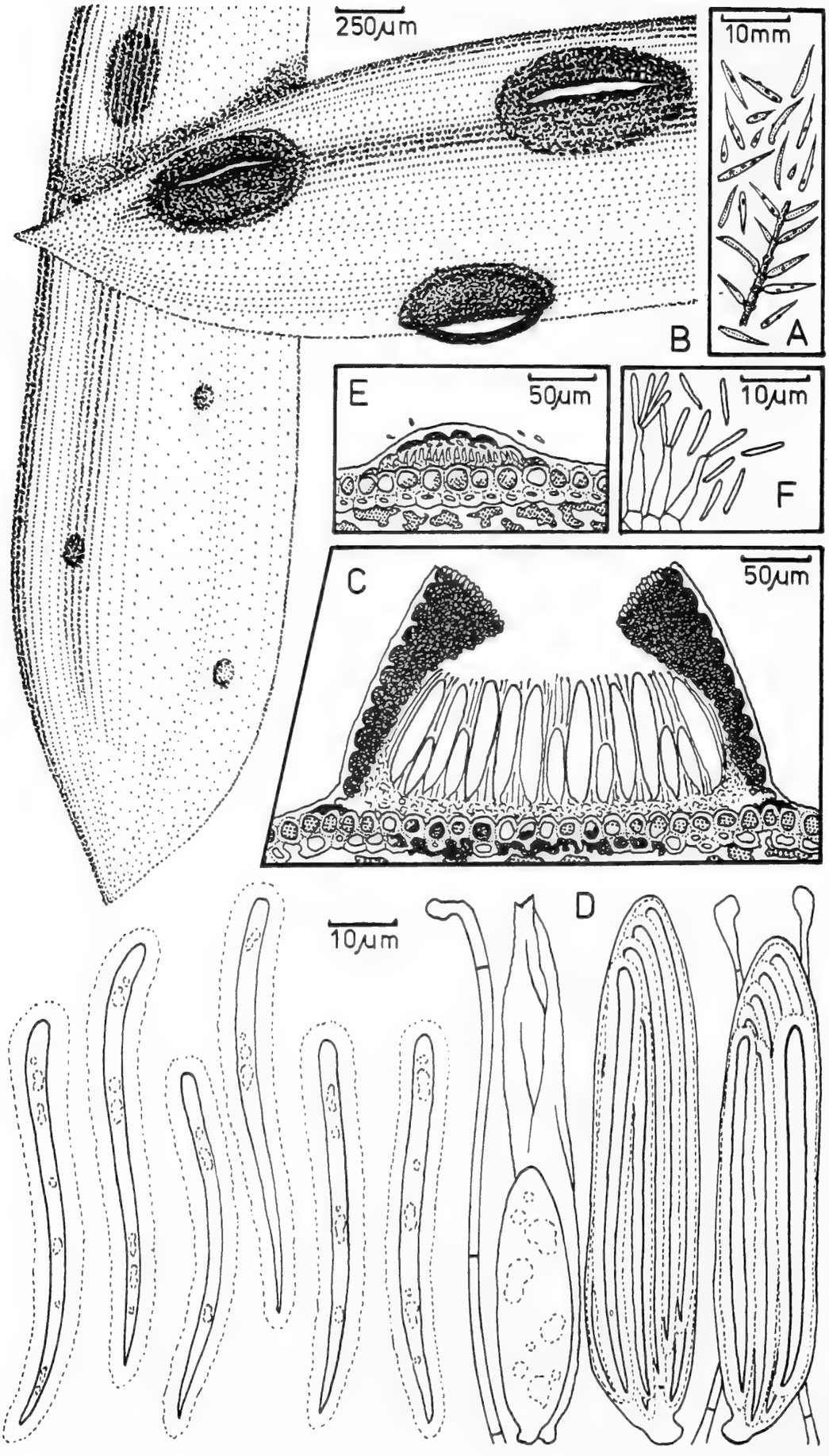

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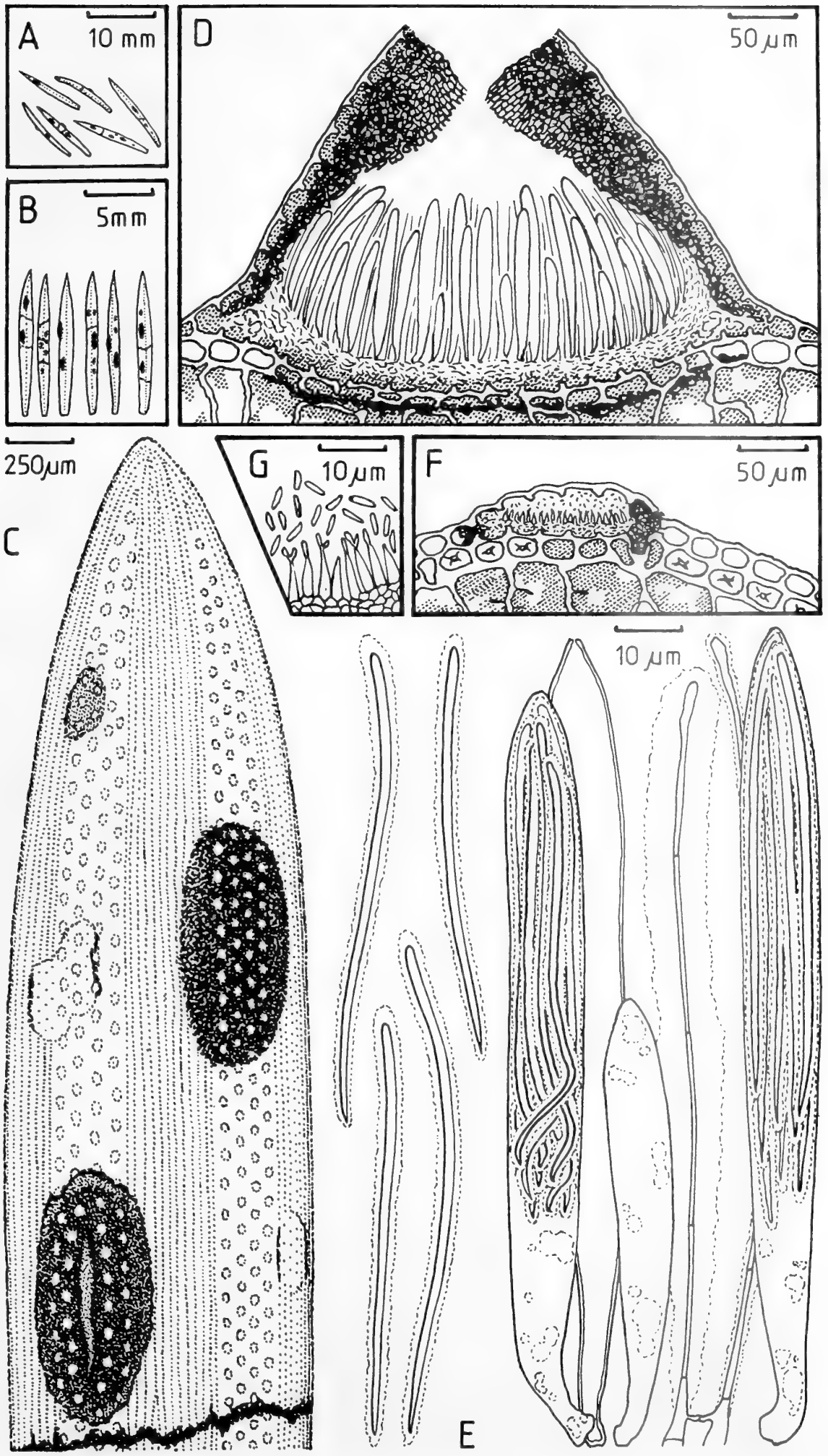
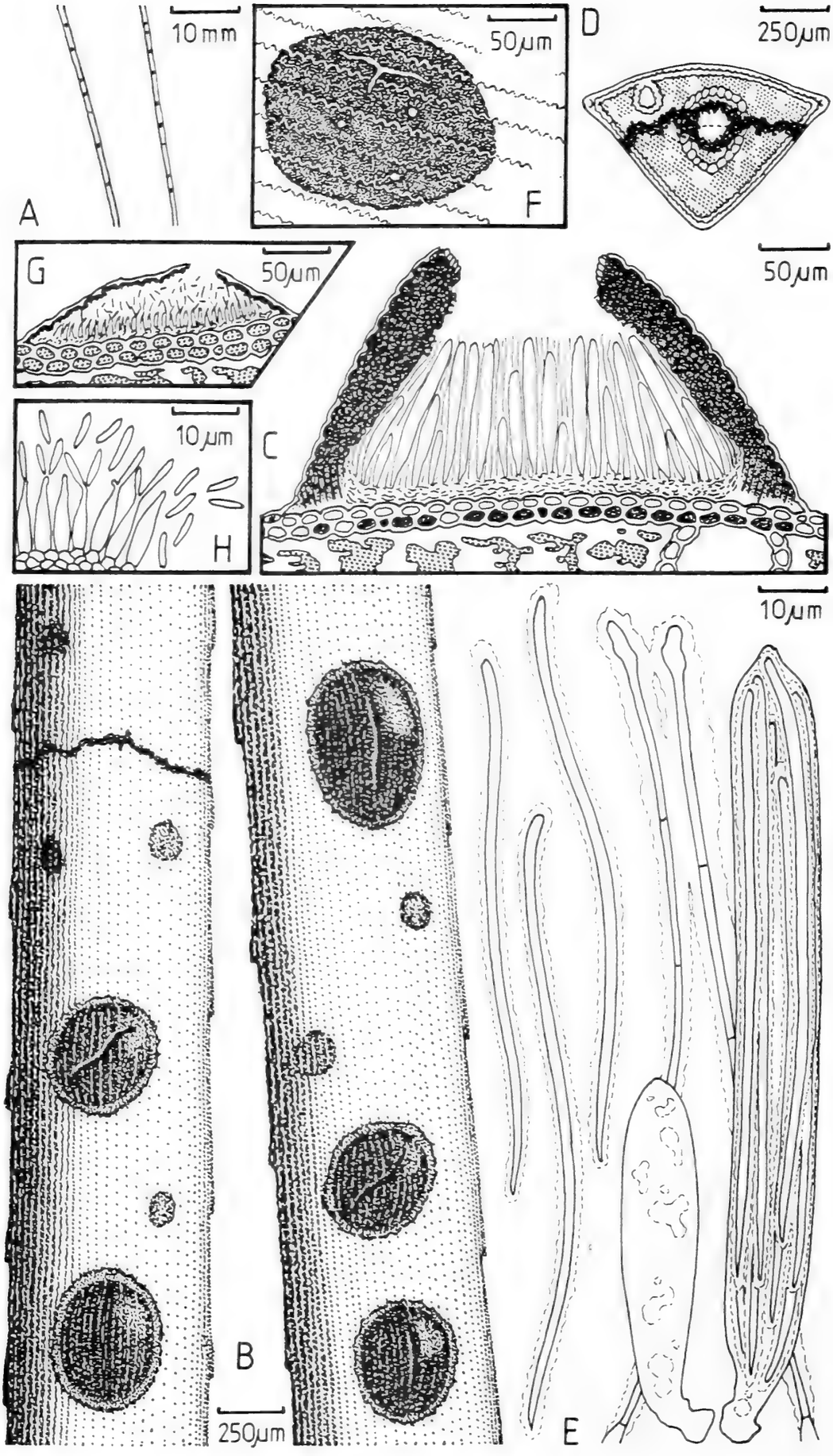
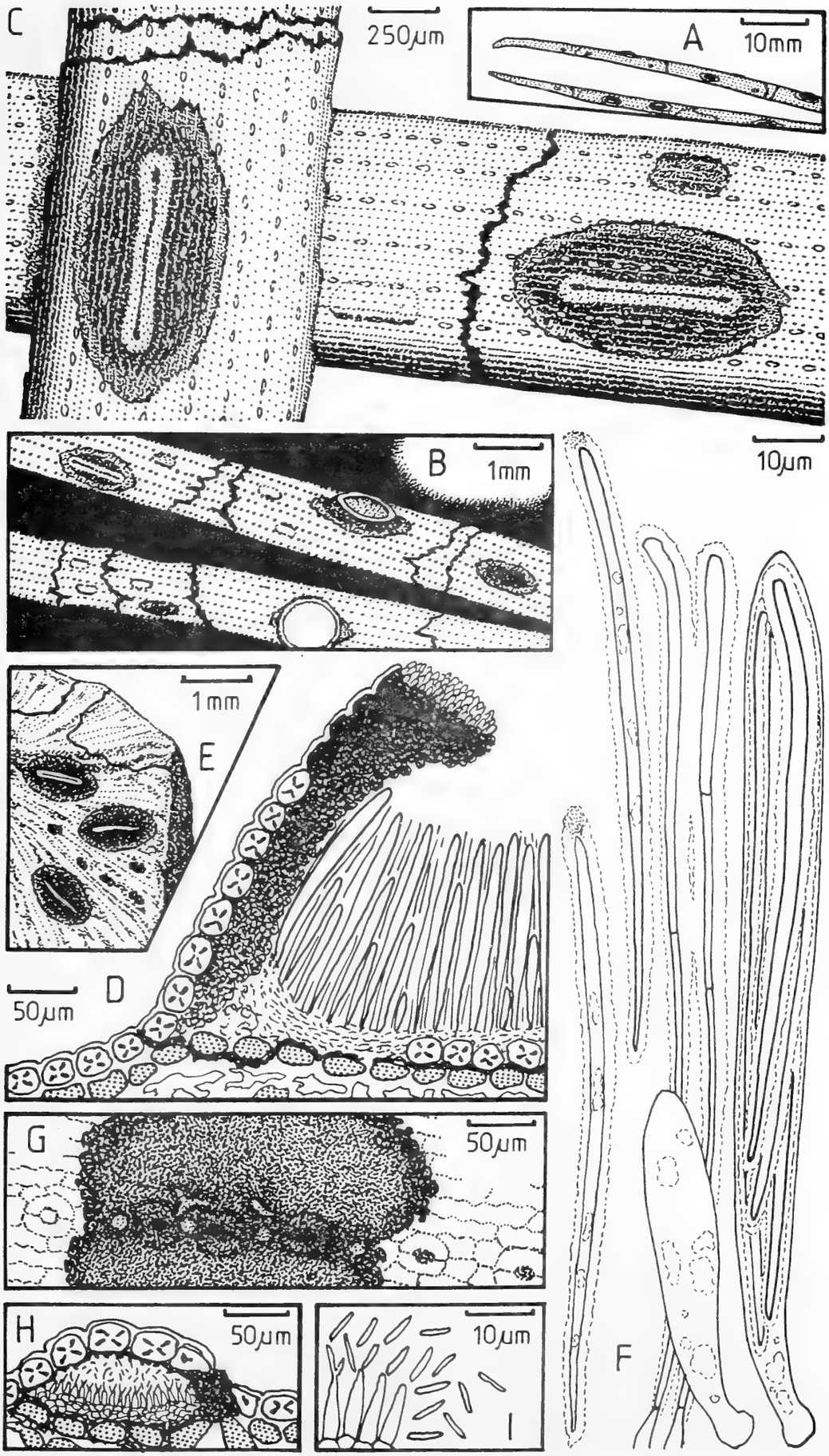


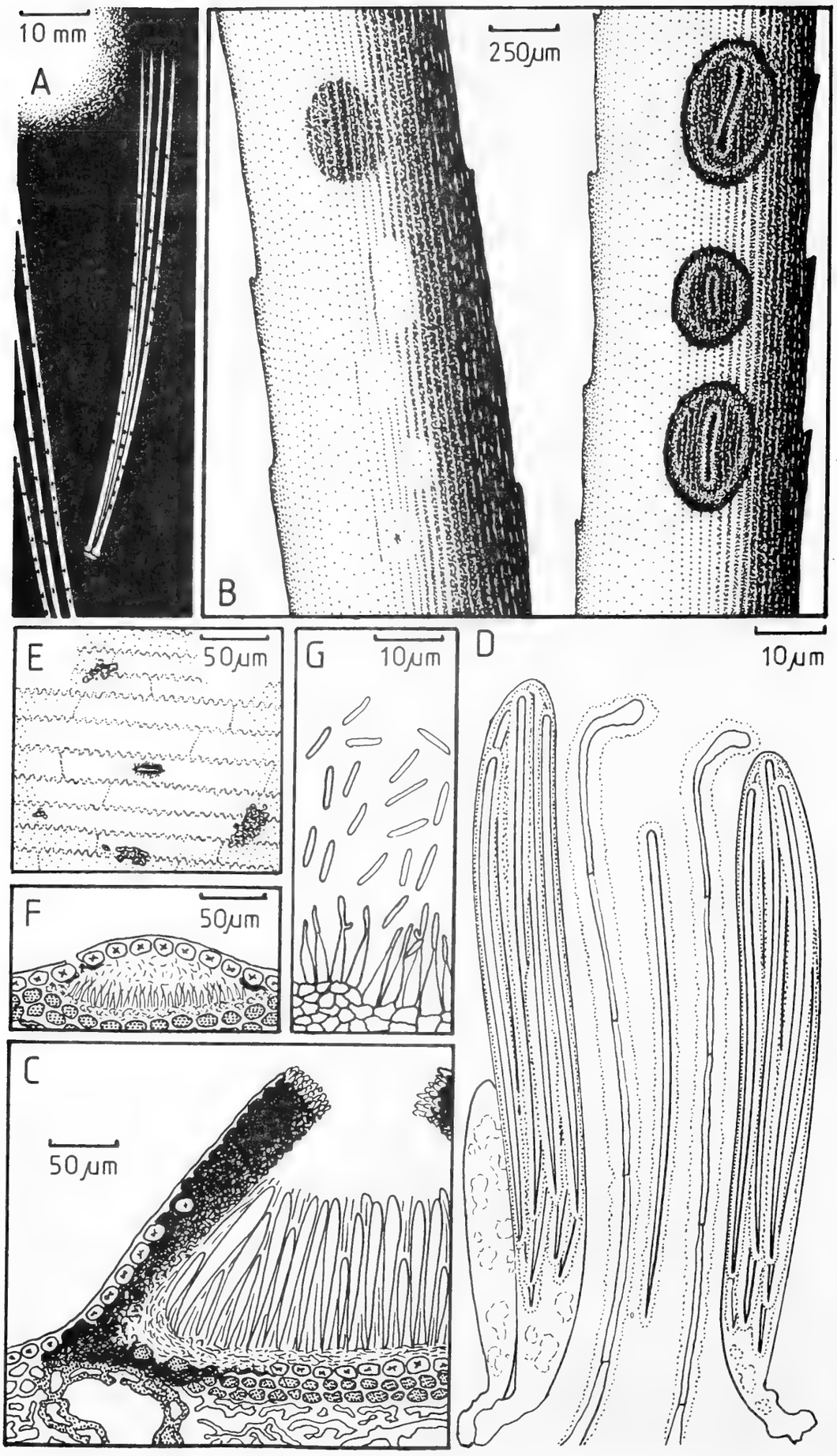
Lophodermium nanakii



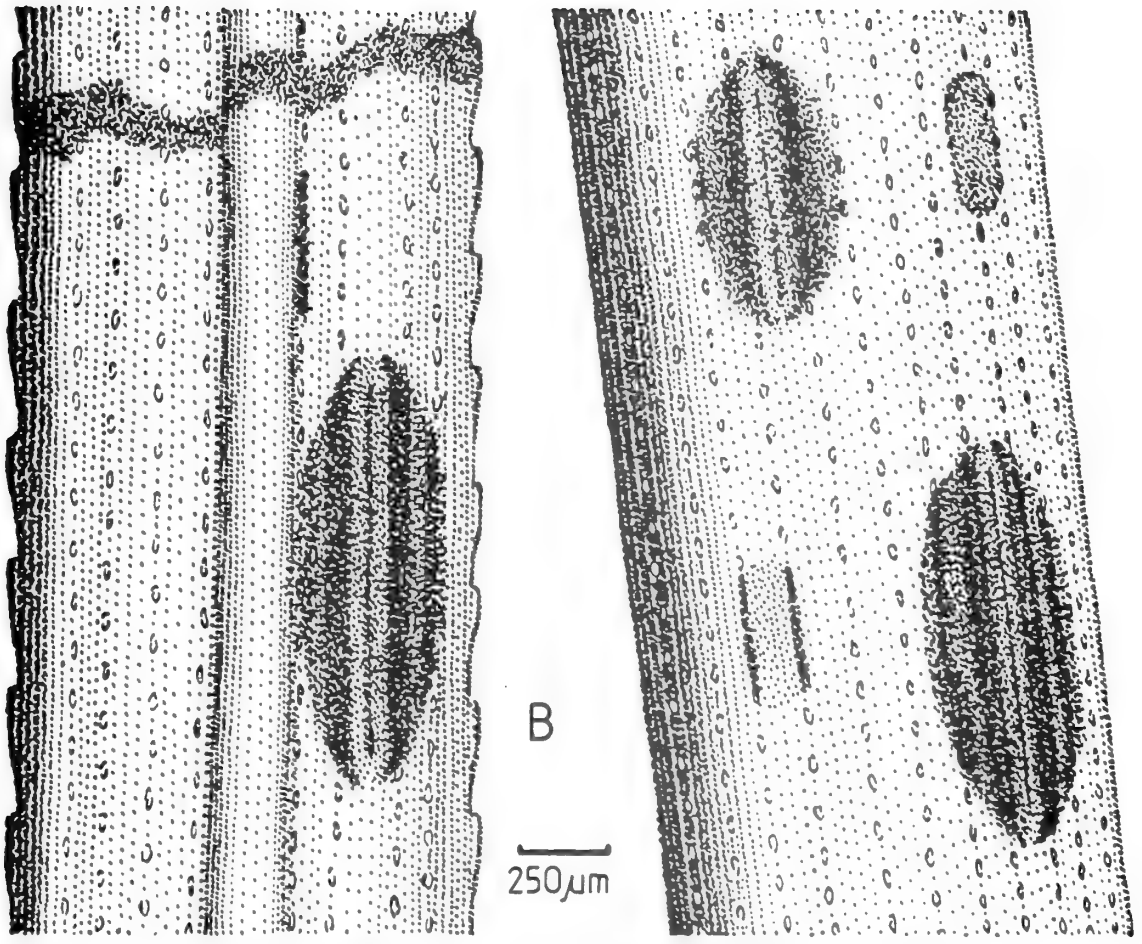
Lophodermium nitens



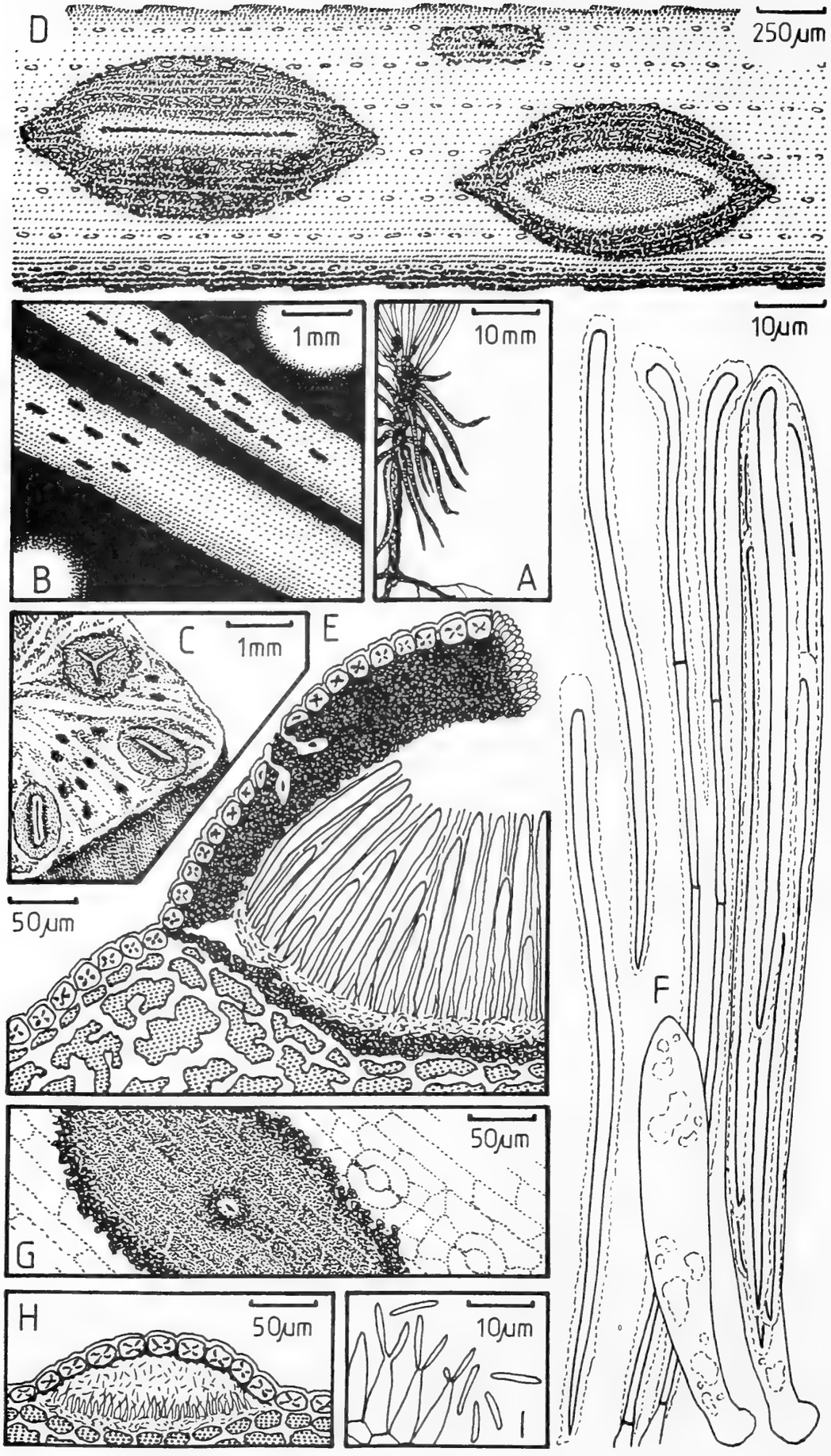
Lophodermium pinastri



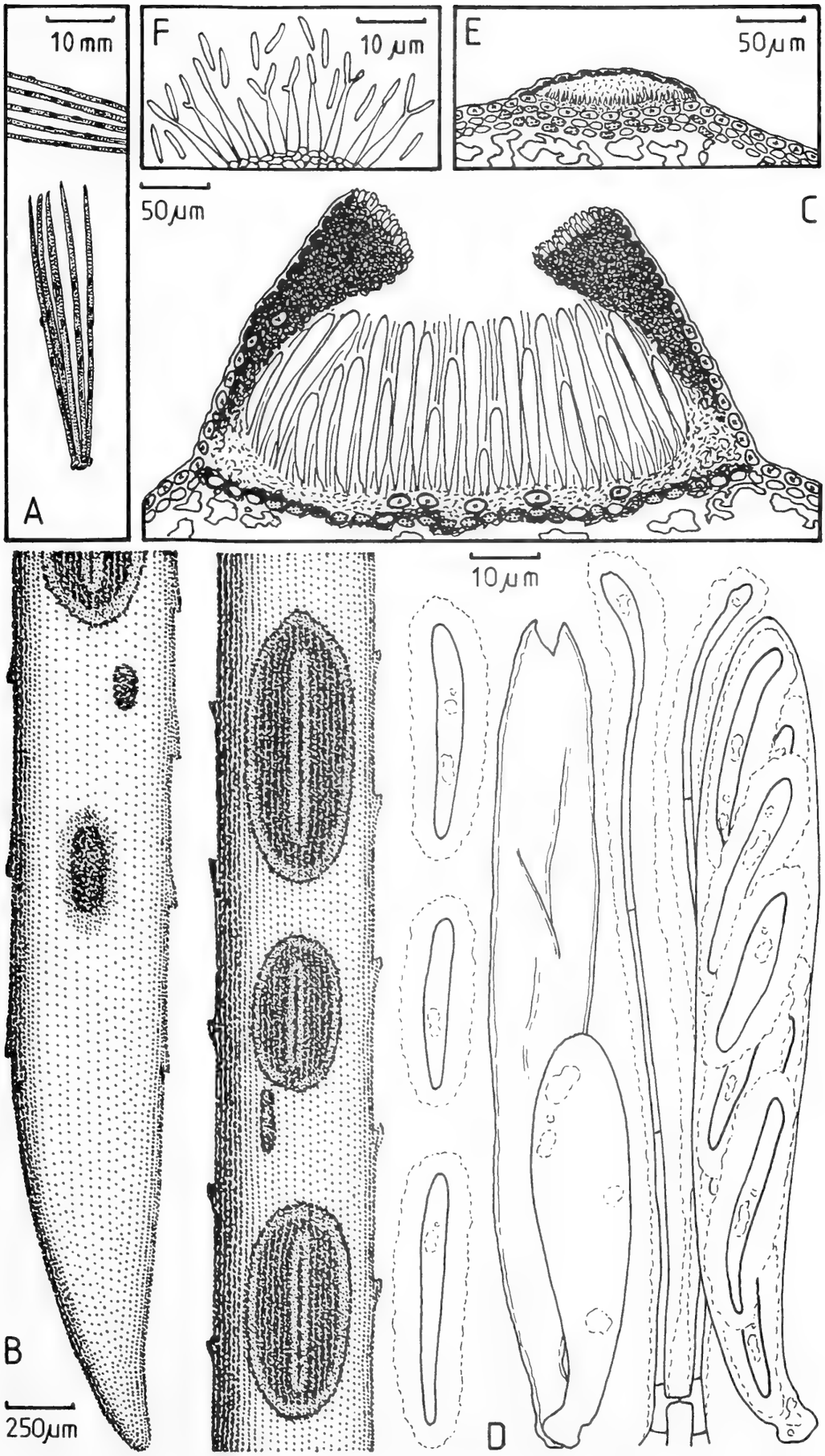
Lophodermium pini-excelsae



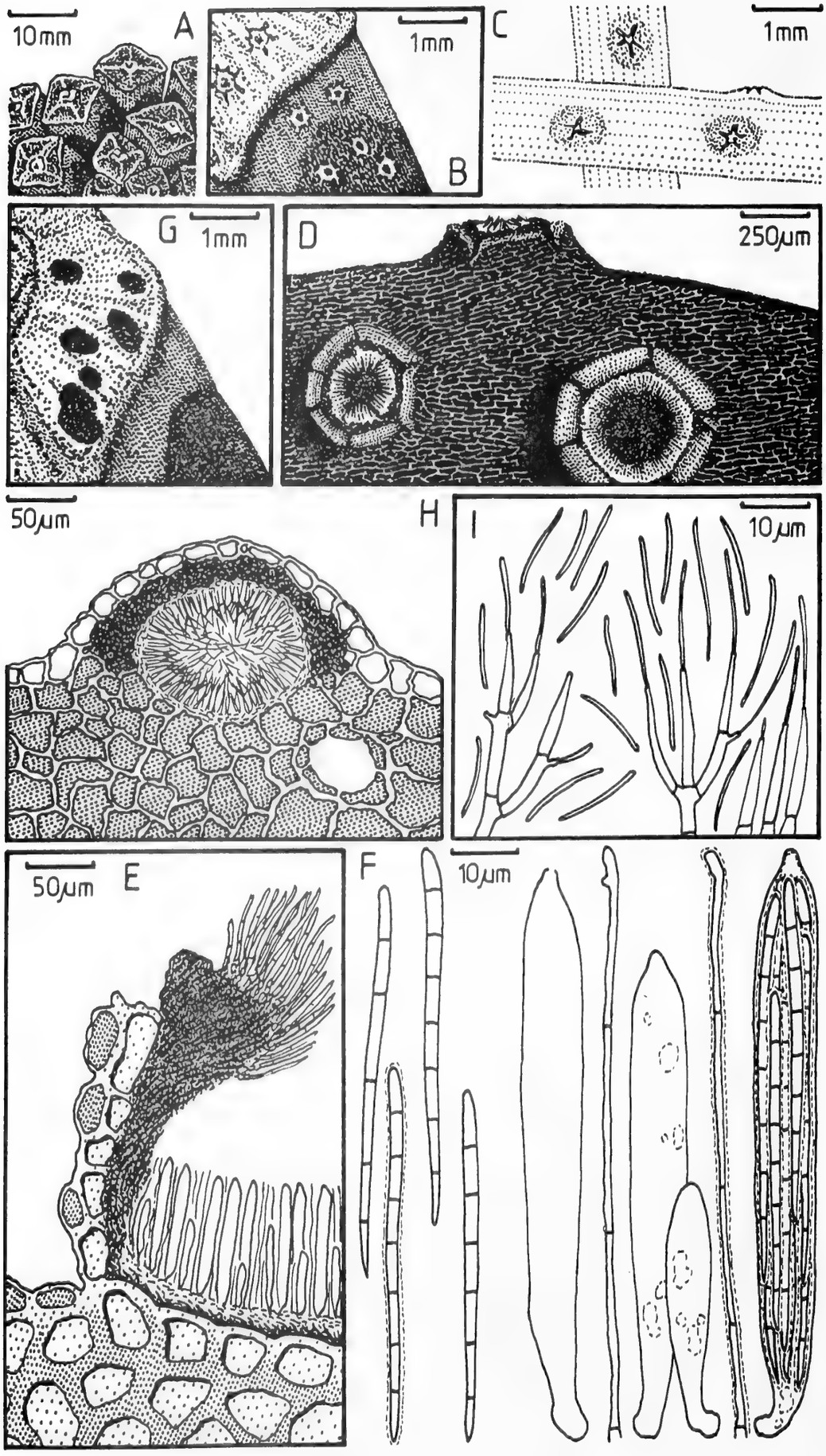
Lophodermium ravenelii



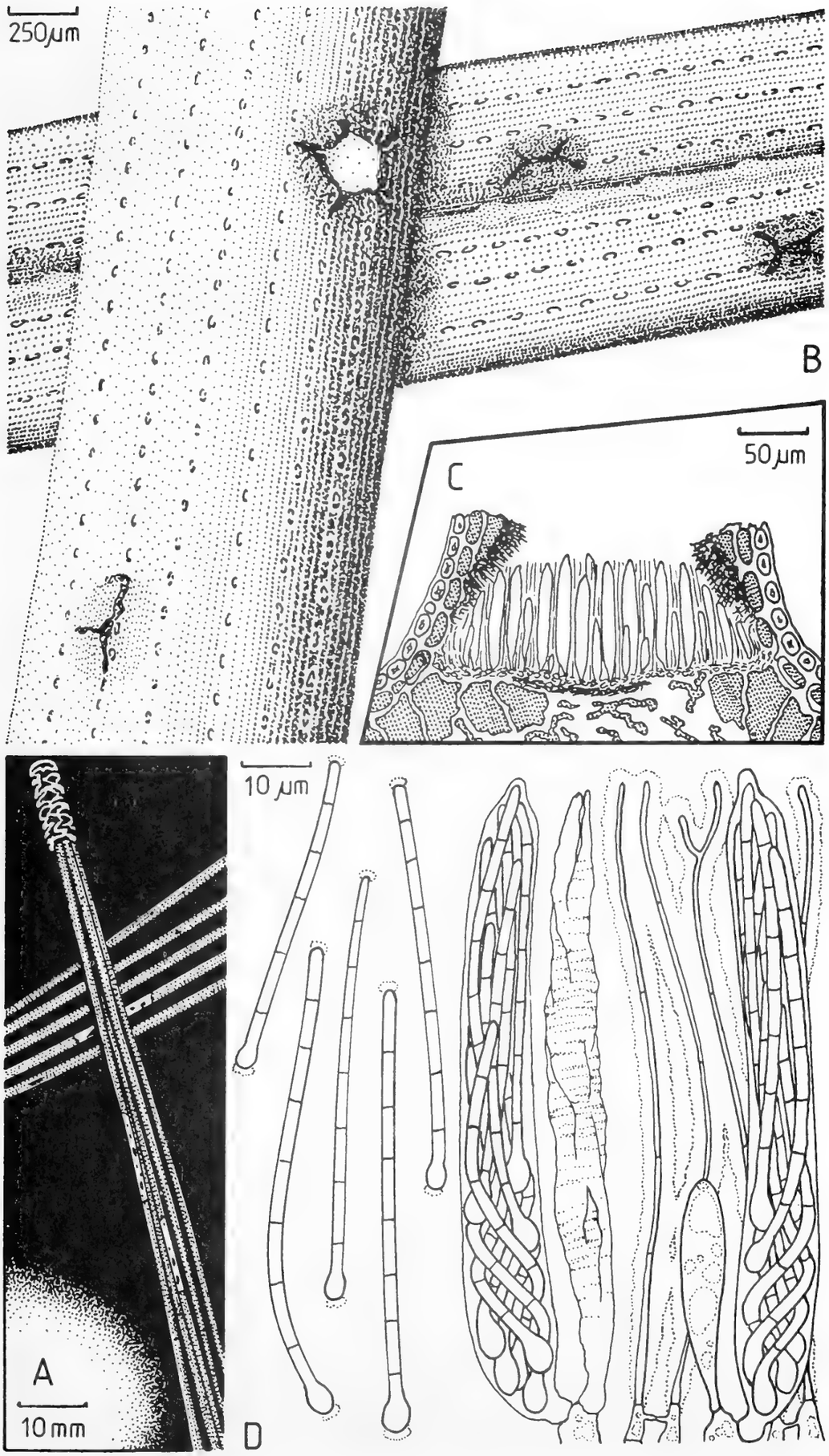
Lophodermium seditiosum



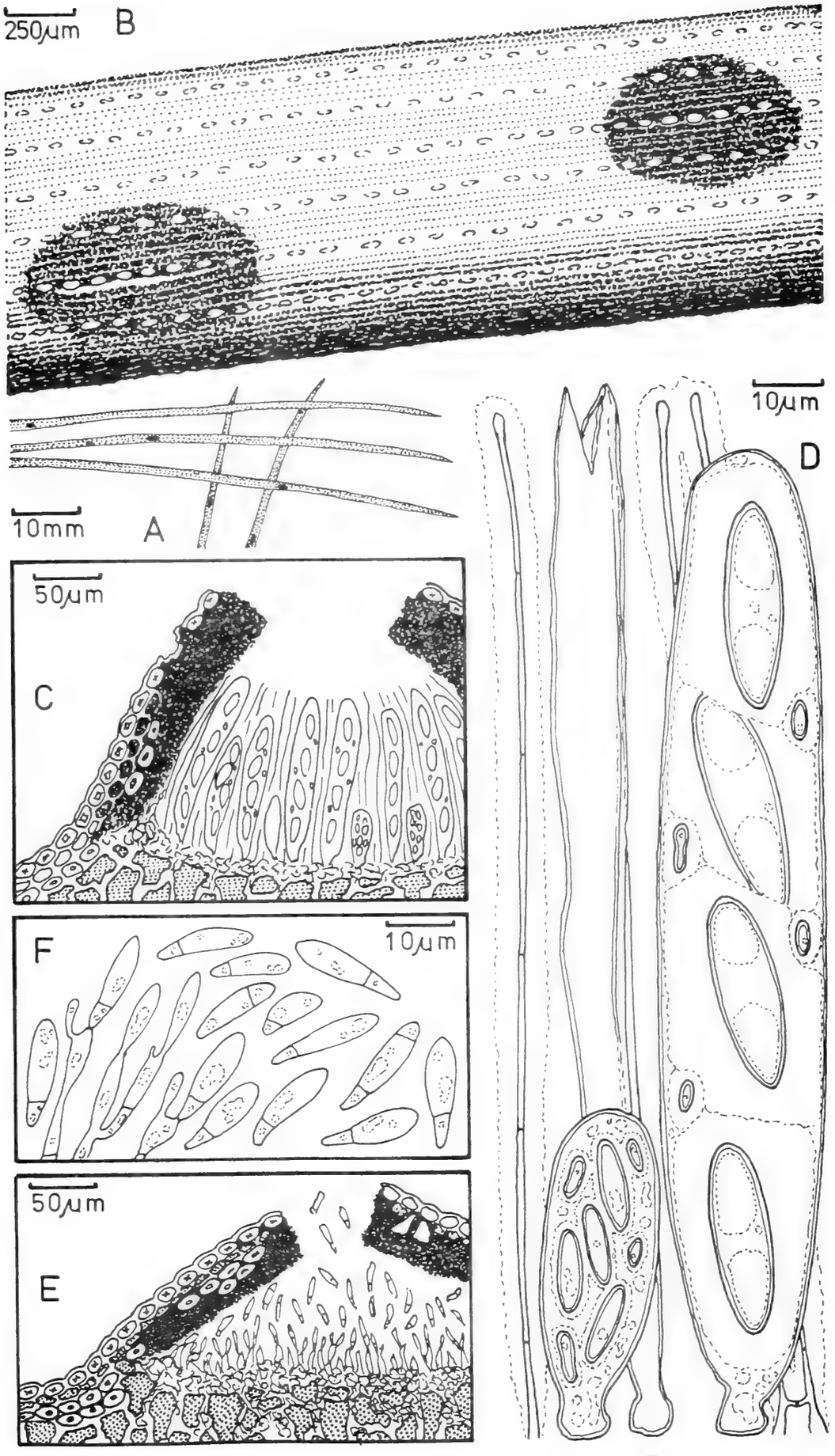
Meloderma desmazieresii



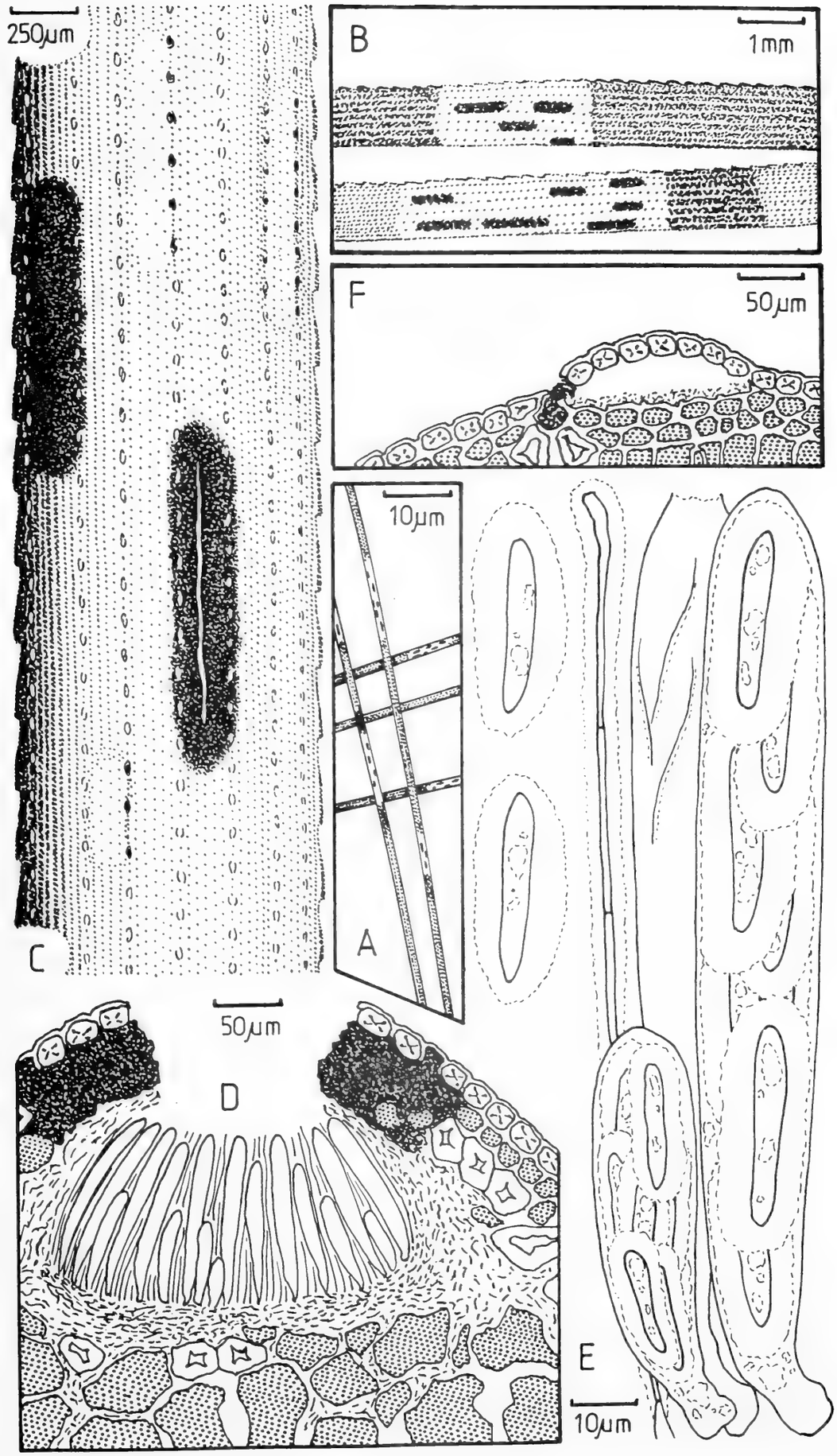
Naemacyclus fimbriatus



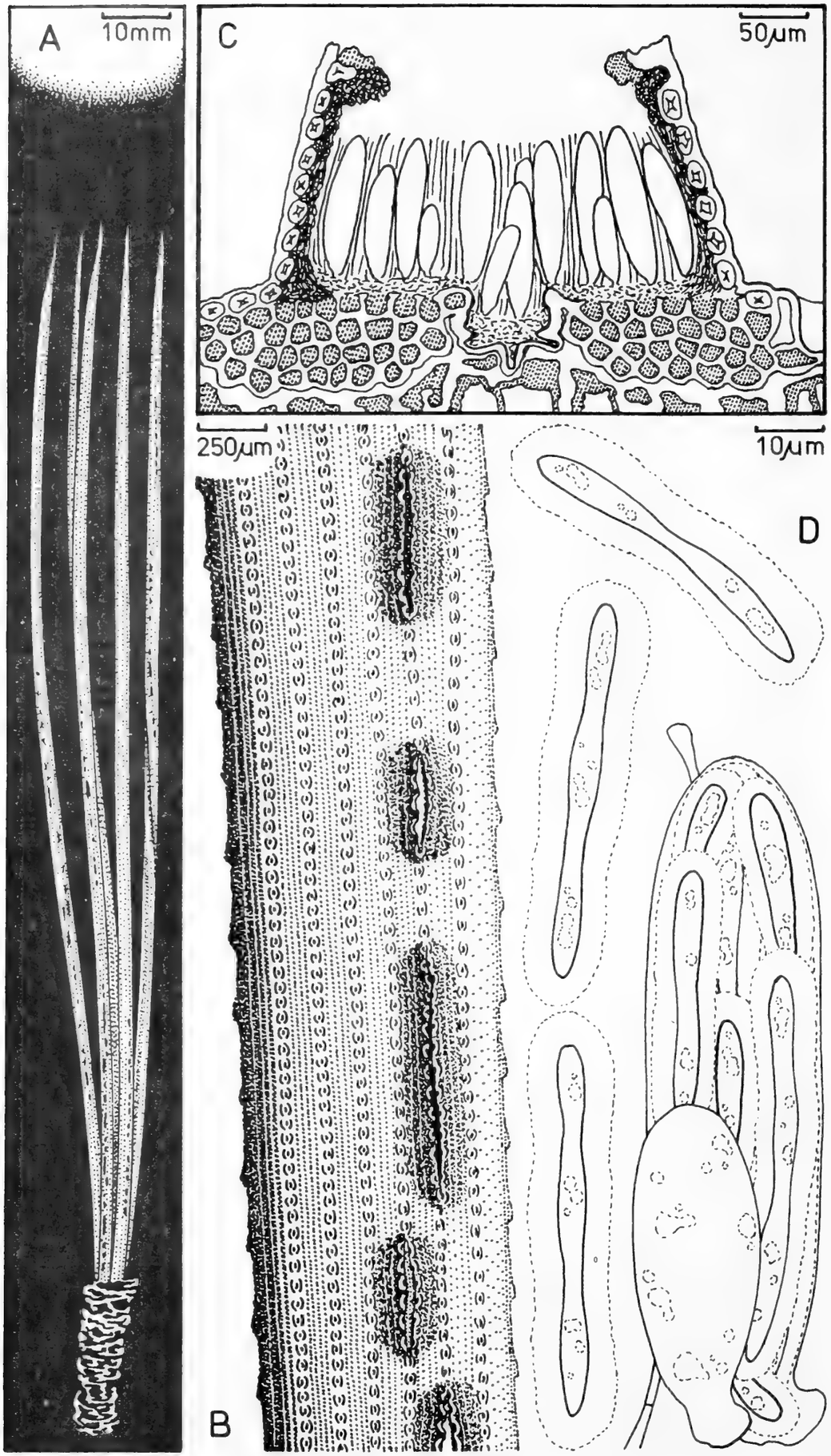
Naemacyclus steatopygioides



Ploioderma hedgcockii



Ploioderma lethale



Soleella striiformis